A Markov model for ranking cricket teams playing one day international matches

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Abstract— Main purpose of ranking teams in One Day International (ODI) Cricket matches is to identify challenging teams of each team. In the sports science literature, various statistical procedures have been proposed by different researchers to rank teams. International Cricket Council (ICC) is using a ranking system as the standard practice. However, it does not work well to realize some head-to head match results. This paper provides a stochastic Markov chain model to rank teams. A comprehensive analysis on head-to-head match prediction for each cricket team is given based on steady-state probabilities. Performance of the proposed model is investigated with ICC ODI rankings. It is shown that the proposed model outperforms to predict results of head-to-head ODI Cricket matches.

Keywords— Stochastic Process, Markov Model, Steady-State Probability, One Day International Cricket ranking

I. INTRODUCTION

The One Day International (ODI) team ranking is an international ranking system authorized by International Cricket Council (ICC) to show the standings of different teams from various nations by analyzing past cricket match performances. The ICC uses a ranking system developed by David Kendix to rank the international full membership teams based on a point system in which, the ranking calculation mechanism gives 66.6% weights for current year matches and 33.3% weightage for matches played in last year played. Past matches have been removed. Currently, there are ten countries namely Sri Lanka, India, Pakistan, Australia, England, South Africa, West Indies, New Zealand, Bangladesh, and Zimbabwe having the full-membership in ICC. In this system, there is a moot point at the rapid incorporation of head-to-head prediction and leads to have difficulties to identify challenging teams for a team. Therefore, it is imperative to develop an acceptable model that works well on head-to-head match prediction.

In this context, our principal goal of this paper was to propose a stochastic model in order to predict head-to-head matches results that could explicitly depict challenging teams for a team. In which, team performance is quantified by total number of wins and losses against opponent teams. An application of the stochastic process in the sport's analysis is not new. Various research papers that predict and analyze game performances in different times using the Markov chain model, have been published. Belman (1976) has introduced a Markov Chain model to Baseball match results. Ursin (2014) have developed a Markov model for baseball with applications. Norman (1999) analyzed the possibilities to use stochastic processes for statistical modeling in sports sciences, especially, Clarke and Norman (1998) utilized the stochastic techniques in various decision-making processes in cricket. Lames (1988) applied the idea of assess the performance of individual players in team games such as tennis as well as Lames and Hohmann (1997) analyzed in volleyball. Zhang (2003) applied this concept for the table-tennis results prediction. Bukiet *et al*. (1997) found optimal batting orders using Markov chain approach in Baseball. Further, Hirotsu and Wright (2003) evaluated the baseball game using the Markov chains. They have expressed how that approach might help to select optimal hitting strategies and how much the probability of winning increases if gained strategy is followed. In addition, winning probability in any state in the game was calculated by using the Markov model. Colwell *et al*. (1991) developed a Markov chain model to outcomes of the test matches between England and Australian teams. They used three states such as team win, lost, and draw. Frequency approach was used to obtain the transition probabilities, where, transition probabilities recorded in 3×3 matrix.

The remaining part of this paper is organized as follows. Section 2 discusses the comprehension of stochastic model, details of proposed stochastic model: how the model was built, how parameters were estimated and examined the model assumptions. In section 3, empirical results of the proposed model are discussed. In the last section, section 4, findings are summarized.

II. METHODOLOGY

A. Comprehension of stochastic model

A stochastic process is a family of random variables, $\{X_t, t\epsilon T\}$, where t usually denotes the time (Jones and Smith 2001). In which, at every time t in the set T, a random number X_t is observed, where the set T is called the index set of the process, might be countable set or an interval of the real line. The transition probability for a Markov chain that jumps from state i to j, P_{ij}; is defined as

$$P_{ij} = \{X_{n+1} = j | X_n = i, X_{n-1} = i_{n-1} \dots, X_1 = i_1, X_0 = i_0\}$$
$$= P\{X_{n+1} = j | X_n = i\}$$

where, $P_{ij} \geq 0,\ i, j \geq 0;\ \sum_{j=0}^{\infty} P_{ij} = 1,\ i = 0,1,2 \dots$

and this type of stochastic process is known as a discrete time Markov Chain (Ross 2010). Every P_{ij} is the row vector for $j = 1, 2, \dots, N$ and these transition probabilities satisfy $\sum_{j=1}^{N} P_{ij} = 1, \forall i$. Markov property, says that, at any time n, the future state X_{n+1} is conditionally independent of the past $X_0, X_1, \dots, X_{n-1}$ and just depend on the present state X_n. In other words, the future behavior of the system depends only on the current state and does not depend on any of the previous states.

The one step transition probabilities of N possible states can be represented by $N \times N$ transition probability matrix P;

$$P = (P_{ij}) = \begin{pmatrix} P_{11} & P_{12} & \dots & P_{1N} \\ P_{21} & P_{22} & \dots & P_{2N} \\ \dots & \dots & \dots & \dots \\ P_{N1} & P_{N2} & \dots & P_{NN} \end{pmatrix}$$

and the matrix $P = (P_{ij})$ is the transition matrix of the chain.

$P_{ij}^{n} = P(X_n = j | X_0 = i)$, is the probability of moving from state i to j in n steps. It can be clearlydescribed by Chapman-Kolmogorov equation as follows;

$$P_{ij}^{n+m} = \sum_{k=0}^{\infty} P_{ik}^{n} P_{kj}^{m} \forall n, m \geq 0 \text{ , all } i \text{ , } j$$

where $P_{ik}^{n} P_{kj}^{m}$ denotes the probability that process moves to state j in $n + m$ transitions starting from state i through a path which take it into state k at the n^{th} transition. Then $P^{n+m} = P^{n} P^{m}$, this equation asserts that $P^{n} = P^{n-1}.P = P^{n}$by induction for $n \geq 1$, where dot (.) denotes matrix multiplication.

A chain is to have a stationary (or steady state) distribution if there exists a vector such that given a transition probability matrix P :

$$\pi = \pi P$$

If a finite Markov chain is irreducible and ergodic then

$$\lim_{n\to\infty} P^{n} = \pi = \begin{pmatrix} \pi_1 & \pi_2 & \dots & \pi_N \\ \pi_1 & \pi_2 & \dots & \pi_N \\ \dots & \dots & \dots & \dots \\ \pi_1 & \pi_2 & \dots & \pi_N \end{pmatrix}$$

where $\pi = (\pi_1, \pi_2, \dots, \pi_N)$ with $0 < \pi_j < 1$ and $\sum_{j=1}^{N} \pi_j = 1$

This stationary probability vector can be viewed as the unique distribution of a random variable in a long-run.

Moreover, steady state probabilities π_j were obtained as

$$\pi_j = \lim_{n\to\infty} P_{ij}^{n} \, \forall i, j \in S.$$

B. Stochastic model building

The objective of this work was to develop a Markov model to rank ODI cricket team's that classify as win or not win in a match. By this classification, it was able to interpret the prediction through Markov chain model. However, the random walk indicates that past data cannot employed to predict the future behavior. Which means n^{th} day team performance is a random process and it is independent from past performances and just depends only immediate past. Hence it is a Markov chain model.

In data collection, secondary data of matches was gathered from 2005 to 2014 from ICC website. The counts of transitions from each state to other state are obtained and the transition probabilities were estimated using maximum likelihood criterion.

To build a Markov chain model, X_n is taken to be the n^{th} day performance of a team and named as

State 1: Team win the match (*W*)

State 2: Team does not win the match (*N*)

So, X_n is a stochastic process that has a value from 1 to 2 on n^{th} day. Moreover, consider team performance in any sequence of letters chosen from the set {*W, N*}. Let n_1, n_2 denote number of letters '*W', 'N*' respectively in the sequence. Let n_{11} denote the number of occurrences in the sequence that the letter '*W*' is immediately followed by the letter '*W*'. Let n_{12}denotes the number of occurrence in the sequence that the letter '*W*' is immediately followed by the letter '*N*'. And n_{21}, n_{22} are similarly defined.

Where, here there are two states and this sequence of results recorded in 2×2 matrix.

$$\begin{array}{c} \\ W \\ N \end{array} \begin{array}{c} \begin{array}{cc} W & N \end{array} \\ \begin{bmatrix} \mathrm{n}_{11} & \mathrm{n}_{12} \\ \mathrm{n}_{21} & \mathrm{n}_{22} \end{bmatrix} \end{array}$$

The transition probability matrix P was formed by dividing each element by the corresponding row total.

$$P = (\hat{p}_{ij}) = \begin{array}{c} \\ W \\ N \end{array} \begin{array}{c} \begin{array}{cc} W & N \end{array} \\ \begin{bmatrix} \hat{p}_{11} = n_{11}/(n_{11+}n_{12}) & \hat{p}_{12} = n_{12}/(n_{11+}\, n_{12}) \\ \hat{p}_{21} = n_{21}/(n_{21+}n_{22}) & \hat{p}_{22} = n_{22}/(n_{21} + n_{22}) \end{bmatrix} \end{array}$$

And also $\sum_{j=1}^{2} \hat{p}_{ij}$=1

This expresses the fact that if the system is in one of the states at one observed value, it will with certainty be in one of the states at the next observed value. For example, *P*(*N, W*) means that probability of not win followed by win. This *(2×2)* matrix of transition probabilities is, called the first step transition probability matrix of the Markov chain. Moreover, each row of *P* is the probability distribution relating to a transition from state *i* to state *j*.

Further, it is checked the stationary (or steady state) distribution and obtained steady-state probabilities π_j as

$$\pi_j = \lim_{n\to\infty} P_{ij}^{n} \qquad \forall i, j \in S.$$

Teams were ranked based on winning probabilities in a long run. Thus, a new ODI Markov chain ranking system has been proposed. In model validation, ICC ODI ranking and MC ODI ranking were cross-validated.

III. RESULTS AND DISCUSSION

Table1: Transition probabilities and steady state probabilities

Team	WW	WN	NW	NN	Steady state probability (π)	
Australia	0.7143	0.2857	0.6216	0.3784	[0.6851	0.3149]
South Africa	0.6864	0.3136	0.6333	0.3667	[0.6689	0.3311]
India	0.5974	0.4026	0.5849	0.4151	[0.5923	0.4077]
Pakistan	0.6239	0.3761	0.4944	0.5056	[0.5680	0.4320]
Sri Lanka	0.5778	0.4222	0.5278	0.4722	[0.5556	0.4444]
New Zealand	0.5904	0.4096	0.4023	0.5977	[0.4955	0.5045]
England	0.5161	0.4839	0.4327	0.5673	[0.4721	0.5279]
Bangladesh	0.5890	0.4110	0.2778	0.7222	[0.4033	0.5967]
West Indies	0.5584	0.4416	0.2857	0.7143	[0.3929	0.6071]
Zimbabwe	0.4318	0.5682	0.2202	0.7798	[0.2793	0.7207]

ICC ODI matches results from January 2005 to March 2014 have been used in this study. The transition probabilities and steady state probabilities of ten test match playing nations are given in Table 1. If Australian team wins a match, then probability that it will win next match is 0.7143. However, if Australian team loses a match, then the probability that team will wins next match is 0.6216, which is less than the probability of win follows by another win (0.7143). Moreover, this behavior can be observed in all ten nations. That is, for any cricket team, chance of a win follows a win is higher than a win follows a loss.

Table 2: Steady state winning probabilities

Team	Steady state winning Probability
Australia	0.685
South Africa	0.668
India	0.592
Pakistan	0.568
Sri Lanka	0.555
New Zealand	0.495
England	0.472
Bangladesh	0. 403
West Indies	0.392
Zimbabwe	0.279

Table 2, shows the steady state winning probabilities of ten teams. Australian team's steady state winning probability is 0.6851, thus, in a long run the probability of winning a match for the Australian team is 0.6851, this is irrespective of results of previous matches. South Africa's steady state winning probability is 0.6689, which is next to Australia. India, Pakistan and Sri Lanka are the succeeding countries in that order. It could be noticed that a ranking among teams which is based on steady state probabilities. The proposed ranking scheme could be called as Markov Chain ODI ranking (MC ODI ranking). Table 3 compares both the existing ICC ODI rankings in March 2014 and the proposed MC ODI rankings.

The existing ICC ODI ranking scheme is based on points, but the proposed Markov Chain ODI ranking scheme is based on steady state probabilities. In prediction, though the point of the existing ranking scheme does not have a straight forward interpretation, steady state probability of the proposed MC ODI ranking scheme has a straight forward interpretation. For instance, as per the existing ranking scheme, Australia's points is 7579, but as per the proposed MC ODI ranking scheme Australia's steady state winning probability is 0.6851. This means in a particular match, Australian team's winning chance is 68.51%.

Table 3: ICC and MC ODI Rankings

Rank	ICC ODI Ranking (March 2014)	MC ODI Ranking
01	Australia	Australia
02	Sri Lanka	South Africa
03	India	India
04	South Africa	Pakistan
05	England	Sri Lanka
06	Pakistan	New Zealand
07	New Zealand	England
08	West Indies	Bangladesh
09	Bangladesh	West Indies
10	Zimbabwe	Zimbabwe

As it was mentioned earlier, principal goal of this paper was to propose a stochastic model in order to predict head-to-head match result that could explicitly depict challenging teams for a team. The steady state probabilities Table 2 and points of the existing ICC ODI ranking both were calculated considering all the matches (irrespective of opponent team) of a team. However, there might be some interaction effects between teams. For instance, south Asian teams could do well against the spin bowling attack. A team which has good spin bowling attack could challenge other teams but may not be able to challenge south Asian teams. Thus, one team might not perform well against a team which is below than that team in ICC ODI or MC ODI ranking. This motivated to do further precise analysis on head-to-head match prediction. Therefore, a detailed further analysis was done for all teams.

Table 4: Transition probability and head-to-head steady state probability of Sri Lankan cricket team

Team	WW	WN	NW	NN	Steady state probability (π)	
India	0.3182	0.6818	0.3947	0.6053	[0.3667	0.6333]
Australia	0.3846	0.6154	0.4500	0.5500	[0.4224	0.5776]
Pakistan	0.6000	0.4000	0.3889	0.6111	[0.4930	0.5070]
South Africa	0.5000	0.5000	0.5000	0.5000	[0.5000	0.5000]
West Indies	0.4000	0.6000	0.7500	0.2500	[0.5556	0.4444]
England	0.6364	0.3636	0.5714	0.4286	[0.6111	0.3889]
New Zealand	0.7857	0.2143	0.5000	0.5000	[0.7000	0.3000]
Bangladesh	0.8095	0.1905	0.9000	0.1000	[0.8400	0.1600]
Zimbabwe	0.8889	0.1111	0.9000	0.1000	[0.9000	0.1000]
India	0.3182	0.6818	0.3947	0.6053	[0.3667	0.6333]

Separate transition probabilities and steady state probabilities were calculated for each team against all opponents of that particular team. As an illustration, consider results of comprehensive analysis of Sri Lankan team. In this respect, separate transition probabilities and steady state probabilities were calculated for Sri Lankan team against each opponent teams. Table 4 provides results of this detailed analysis. Table 5 shows separate steady state winning probabilities of the Sri Lankan team against each opponent teams. From Table 1 Sri Lankan team's common (irrespective of opponent team) steady state winning probability is 0.5556. However, in

head-to-head analysis, summarized results in Table 5 say that Sri Lankan team's steady state winning probability heavily depends on opponent team. The steady state probabilities vary with opponent team, ranging from 0.366 to 0.9. As per these results, steady state wining probability of the Sri Lankan team is less than 0.5 against India, Australia, and Pakistan and steady state probability is 0.5 against South Africa. For the rest of the teams Sri Lankan team's wining probability is greater than 0.5. Thus, India, Australia and Pakistan are the challenging teams for the Sri Lankan team.

Table 5: Head-to-head steady state winning probability for Sri Lankan team

Opponent Team	Steady state winning probability for Sri Lanka
India	0.366
Australia	0.422
Pakistan	0.493
South Africa	0.500
West Indies	0.556
England	0.611
New Zealand	0.700
Bangladesh	0.840
Zimbabwe	0.900

In the model validation in predictions, it could be used both existing ICC ODI ranking and head-to-head winning steady state probabilities of Table 5. Australia is above Sri Lanka in both rankings, that is, Australia is a challenging team to Sri Lankan team in both ranking methods. However, as per the ICC ODI ranking scheme India and Pakistan are below Sri Lankan team and they are not challenging teams to Sri Lanka. But, as per the proposed Markov chain based head-to-head prediction both India and Pakistan are challenging teams for Sri Lankan team. Therefore, future ODI match results of Sri Lanka versus India and Sri Lanka versus Pakistan could be used to validate the proposed head-to-head ranking method.

The ODI matched results from March 2014 to December 2017 has been used as a test bed to validate the proposed ranking method. There were 14 head-to-head matches between Sri Lanka and India during the above period. As per the ICC ODI ranking Sri Lanka is the favorite and as per the MC head-to-head prediction India is the favorite. Among those 14 matches India has won 12 matches and Sri Lanka has won only 2 matches. Thus, match result prediction based on ICC ODI ranking was correct two times and the proposed MC based prediction was correct on twelve times. Similarly 14 head-to-head matches played between Sri Lanka versus Pakistan was also considered as another test bed. As per the prediction based on ICC ODI ranking Sri Lankan team is the most favorite but as per the prediction based on MC, Pakistan is the most favorite. Among those 14 matches, Sri Lanka has won only 3 and Pakistan won 11 matches. Here, again MC based head-to-head prediction worked better than ICC ODI based prediction in finding challenging teams.

Similarly, a detailed comprehensive further analysis was carried out for the rest of teams and MC based head-to-head prediction steady state probabilities were obtained. It could be noticed that except West Indies versus Bangladesh all other MC based head-to-head predictions exactly matched with ICC ODI ranking based prediction.

As per ICC ODI ranking West Indies is the favorite team for head-to-head prediction with Bangladesh team (see, ICC ODI ranking in Table 3). However, steady state winning probability for West Indies against Bangladesh is 0.4375, which is less than 0.5. Thus, as per the MC based head-to-head prediction, Bangladesh is the most favorite team. Here, again three ODI matches between West Indies and Bangladesh played between March 2014 and December 2017 have been used as the test bed. Bangladesh has won all three ODI matches. Thus, MC based head-to-head prediction worked better than ICC ODI based prediction. Table 6 summarizes the cross-validation significant results on our test beds.

IV. CONCLUSION

The ICC ODI ranking system is suspected to provide the reliable realization on some head-to-head match result for a person who is fascinated in Cricket. So, this paper proposed a new head-to-head prediction method so-called Markov chain based head-to-head prediction that can more effectively describe challenging teams for each team in ODI Cricket matches. To show the superiority of the proposed model with the ICC ODI ranking system, it was used real-data from 2005 to 2014 (March) for model fitting and 2014 (April) to 2017 for the model validation. In which, teams were ranked based on steady state probabilities of each team respective to opponent teams, and head-to-head proposed model working efficiency was investigated by the cross-validation with ICC ranking. The proposed model outperforms well than ICC to predict head-to-head matches in ODI cricket matches.

ICC ODI ranking scheme is based on points, but, in prediction, those points do not have a straight forward interpretation, however, steady state probabilities of the proposed MC ODI ranking scheme has a meaningful interpretation. It is believed that this proposed ranking system can be developed to predict the cricket players' performances against each team as well as teams in the international test Cricket matches.

Table 6: Significant results

Team	Opponent	ICC ODI ranking favorite	MC head-to- head prediction favorite	Results	Percentage of correct Prediction	
					In favor of ICC ODI ranking	In favor of MC head-to-head prediction
Sri Lanka	India	Sri Lanka	India	India won 12 out of 14 Matches	14.29%	85.71%
Sri Lanka	Pakistan	Sri Lanka	Pakistan	Pakistan won 11 out of 14 matches	21.43%	78.57%
West Indies	Bangladesh	West Indies	Bangladesh	Bangladesh won all 3 matches	0%	100%

V. REFERENCES

Bellman, R. (1976). *Dynamic Programming and Markovian Decision Processes, with Application to Baseball*, Departments of Electrical Engineering, Mathematics and Medicine, UCLA. 12A(6), pp. 633-651

Bukiet, B. Harold, E and Palacios, J. A. (1997). *Markov chain approach to baseball Operations Research* 45(1), pp.14-23

Clarke, S. R. and Norman, J.M (1998). *When to rush behind in Australian Rules football: a Dynamic Programming approach*, Journal of Operational Research Society, 49(5), pp.530-536

Colwell, D. Jones, B. and Jillet, J. (1991). *A Markov Chain in Cricket*. *The Mathematical Gazette*, 75, pp.183-185.

Hirotsu, N. and Wright, M. (2003). *A Markov chain approach to optimal pinch-hitting strategies in a designated hitter rule baseball game*, Journal of the Operations Research Society of Japan, 46, pp.353-371.

Jones, P. and Smith, P. (2001). *Stochastic processes an introduction*, Arnold texts in statistics, London: Arnold, 7, pp.259

Lames, M. (1988). *Sportspiele: animieren – trainieren*, Techniktraining im Tennis durch Computer simulation In R. Ahrensburg: Czwalina, pp.181-191.

Lames, M. and Hohmann, A. (1997). *Integrative Aspekte in Theorie und Praxis der Rückschlagspiele*. Zur Leistungsrelevanz von Spielhandlungen im Volleyball, pp. 121-128. In B. Hoffmann and P. Koch (Ed.) Hamburg: Czwalina.

Norman, J. M. (1999). *Markov process applications in sport*. In IFORS conference. Beijing, China, 50, pp. 536-545.

Ross, S. M. (2010). *Introduction to Probability Models*, 10thedn. Academic Press

Ursin, D. J. (2014). *A Markov model for baseball with applications*, Thesis and Dissertation. University of Wilconsin-Milwaukee, pp.964

Zhang, H. (2003). *LeistungsdiagnostikimTischtennis*, Doctoral dissertation. University of Potsdam.

Selecting and optimizing a reliable DNA extraction method for isolating viral DNA in okra (*Abelmoschus esculentus*)

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Abstract— Detection and identification of viruses in okra plant is important to manage viral diseases. Good quality of DNA is essential for the PCR based detection and subsequent genomic sequencing. However, the DNA extraction has been greatly affected by mucilaginous substances present in okra plant parts. Objective of this study is to find out an extraction method that can eliminate mucilaginous materials and yield good quality DNA. Five different protocols were tested with okra leaf samples having okra yellow vein mosaic disease (OYVMD) symptoms. Quantity and purity of extracted DNA was tested using Nanodrop spectrophotometer. Precise quantity comparison was done by measuring total plant DNA in relation to the copy number of *ACT2* gene using quantitative polymerase chain reaction (qPCR). The extracted DNA samples were used as template for detection of *Bhendi yellow vein mosaic virus* and *Bhendi yellow vein mosaic betasatellite* using specific primers. A modified protocol (modified method 1), introduced in this study, produced better yield and quality of DNA compared to other tested protocols. The method yielded 32 µg DNA for 100 mg leaf powder and the ratios A260/A280 and A260/A230 were 1.8 and 2.1, respectively. The qPCR quantification further confirmed higher quantity of DNA yield in this modified protocol. End point PCR with virus specific primers yielded very bright bands for *Bhendi yellow vein mosaic virus* and *Bhendi yellow vein mosaic betasatellite*. In conclusion, the modified method 1 can be used to extract good quality and quantity of DNA from okra.

Keywords— Okra, DNA extraction, plant viruses

I. INTRODUCTION

Detection and identification of plant viruses is the basis to manage plant diseases and to predict the crop loss by the infection. There are various approaches to detect and identify the viruses associated with diseased plants. Nucleic acid-based diagnostic techniques such as hybridization, amplification of targeted viral genome or genomic component(s) by the polymerase chain reaction (PCR) and DNA sequencing have overwhelmingly replaced the use of microscopical and serological techniques (Jose and Usha, 2000; Sharma *et al.*, 2005; Khan *et al.*, 2007; Kushwaha *et al.*, 2010). Nucleic acid based diagnostic techniques are more reliable, specific and reproducible. Better quality and enough quantity of virus genomic DNA or RNA are pre-requisite since the genomic components of the viruses are mixed with cellular constituents of infected plant tissues (Swanson *et al.*, 1992).

DNA extraction from some plant tissues remains difficult due to the contamination of secondary metabolites (Cavallari *et al.*, 2014). Plants such as Okra (*Abelmoschus esculentus*), Mesta (*Hibiscus cannabinus*), Jute (*Corchorus* sp.) and Sida (*Sida* sp.) possess large amounts of viscous mucilage, which often have secondary metabolites, such as polysaccharides, phenolic compounds, tannins, and alkaloids (Höfer *et al*., 1997; Jose and Usha 2003; Ghosh *et al*., 2008; Roy *et al*., 2009). The viscous mucilage co-precipitates with DNA during total DNA extraction and reduces the quality of the extracted DNA. In addition, attempt to remove the mucilaginous substances always associated with reduction in total DNA quantity (Bayer *et al*., 1999). The mucilage contamination makes the DNA unmanageable during pipetting and interferes with further downstream applications, such as PCR or other enzymatic reactions (Fang *et al.*, 1992). The concentration and viscosity of mucilage varies from one plant species to other or among different cultivars of one species is due to the chemical complexity and variation in the physiological properties (Muralikrishna *et al.*, 1989; Kawamura *et al.*, 2000; Axel Diederichsen *et al.*, 2006)

Finding out a rapid, simple and reliable DNA extraction protocol is most vital in PCR based diagnosis of virus diseases in plants. Cetyl trimethyl ammonium bromide (CTAB) method is one of the most common methods for DNA extraction from plant samples (Doyle and Doyle, 1987). However, a CTAB extraction procedure does not guarantee the elimination of some contaminants such as lipids, phenolic compounds, and viscous polysaccharides (Pedersen *et al.*, 2006). There are several modified versions of CTAB extraction protocols available to extract DNA from virus infected plant tissues (Deng *et al.*, 1994; Mansoor *et al.*, 1999; Rothenstein *et al.*, 2005; Briddon and Markham, 1994).

Jose and Usha (2000) described a protocol for the extraction of geminiviral DNA from infected okra. This protocol is a combination of a modified CTAB method of Dellaporta *et al.* (1983) and a citrate buffer extraction with alkali-lysis. However, the method takes too long to get the extracted DNA.

Ghosh *et al*. (2009) reported that the DNA extracted by standard CTAB method (Doyle and Doyle, 1987) did not respond to PCR or exhibit inconsistent amplification of virus DNA due to the contamination of mucilaginous substances. Even though the isolated DNA was further purified via DNeasy mini spin column (QIAGEN, GmBH, Hilden, Germany) the PCR amplification was not successful. Therefore, Ghosh *et al*. (2009) developed a modified CTAB protocol to extract DNA from highly mucilaginous plant samples. The newly developed method gave enough quantity of DNA to Jute plant. However, this method has few drawbacks, such as utilizing large volume of reagents, time consuming and deals with toxic phenol.

The present study describes an improved, rapid, safe and inexpensive, modified method for DNA isolation which is suitable for further downstream amplification of full or partial genome of begomoviruses and betasatellites. The method described in this study has been standardized with okra plants infected with a begomovirus, *Bhendi yellow vein mosaic virus* (BYVMV) and *Bhendi yellow vein mosaic betasatellite* (BYVMB).

II. MATERIALS AND METHODS

A. Collection of plant samples

Young Leaf samples showing yellow vein mosaic symptoms were collected from three months old okra plants (Variety TV-8) growing in research field, Agriculture research station, Thirunelveli, Jaffna, Sri Lanka in May, 2015. Symptomless leaf samples were collected from plants which were grown in insect-proof cages. The leaf samples were dried by keeping inside paper towels.

B. DNA extraction

DNA extraction was done using above leaf samples. Fine powders of the leaf samples were obtained separately by grinding a whole leaf in liquid nitrogen using pre-chilled sterilized mortar and pestle. The aliquots (20 mg) of the fine powder were taken in 1.5 ml Eppendorf tubes separately. Total DNA was extracted using five different protocols including two modified methods which were introduced in this study (Table 1).

Table 1: DNA extraction protocols used in the study

No.	Protocol	Source
1	Cetyl trimethylammonium bromide (CTAB) method	Doyle and Doyle (1987)
2	An improved method of DNA isolation for mucilaginous plant	Ghosh *et al.* (2009)
3	DNeasy plant mini kit	QIAGEN, Germany
4	Modified method I	Proposed in the current study
5	Modified method II	Proposed in the current study

The protocols 1-3 were carried out as described by the authors or as in manufacturer's guide line and the protocol 4 and 5 are given below. All the chemicals used in the extraction procedures were purchased from Sigma-Aldrich, USA. All the extraction protocols were tested twice.

Protocol 4: Modified method I

This method was developed by modifying the method described by Ghosh *et al*. (2009). An aliquot of 5 ml of extraction buffer (100mM Tris–HCl, 10mM EDTA, 1.4M NaCl, 2% CTAB, 0.2% β-mercaptoethanol and 1% Polyvinylpyrrolidone (PVP), pH 8) was taken in 15 ml falcon tube and pre-warmed at 65 °C. 20 mg of leaf powder was added into the pre-warmed buffer and incubated at 65 °C for 30 min. During incubation period, the suspension was mixed 4 to 6 times by inverting the tubes gently. For removing the organic contaminants, 0.6 volume of chloroform:iso-amylalcohol (24:1) was added into the tube and mixed thoroughly for 1-2 min, to form an emulsion. The samples were centrifuged at 4000 × *g* for 10 min at room temperature and the upper aqueous phase was transferred to a fresh tube. Two-thirds of volume of cold iso-propanol was then added and mixed well by inverting the tube gently. The nucleic acid was collected as pellet by centrifuging at 4000 × *g* for 10min. The pellet was air-dried and re-suspended in 1 ml of TE buffer. The solution was transferred to a fresh 2 ml micro-centrifuge tube and treated with RNase at 37°C for 10 min in heating block. Then equal volume of chloroform: iso-amylalcohol (24:1) was added and mixed thoroughly to form an emulsion. The samples were centrifuged at 16,500 × *g* for 5 min at room temperature and the upper aqueous phase was transferred to a fresh tube. A double volume of absolute ethanol was added into the above aqueous phase and mixed gently by inverting the tube to precipitate the DNA. After centrifugation at 4000 × *g* for 10 min at 4 °C, the DNA pellet was washed with washing buffer, composed of ethanol (76%) and ammonium acetate (10 mM). Finally the air-dried DNA pellet was dissolved in 75 µl of TE buffer.

Protocol 5: Modified method II

The second modified method was developed by combining CTAB method and commercial DNA extraction kits. The procedure was similar to protocol 4 up to precipitation of nucleic acid by adding chilled iso-propanol. The resulting pellet was used for further extraction using DNeasy plant mini-kit using manufacturer's instruction.

C. Measuring the quality and quantity of extracted total DNA

To measure the DNA concentration, 1.5 µl of extracted DNA of each sample was loaded in Nanodrop spectrophotometer (Nanodrop 2000, Thermo scientific) and the concentration was measured at 260 nm wave length. The quality of the extracted DNA was measured based on the reading that obtained from A260/A280 nm and A260/A230 nm.

D. Quantification of plant DNA in the extracted total DNA using qPCR

The plant DNA in each extracted DNA sample was quantified and compared in relation to amount of plant actin gene. Pair of primers ACT2 F and ACT2 R (Table 2) amplifying a 68-bp genomic fragment of the *ACT2* gene (Che *et al*., 2010) was used in this experiment.

Quantification was done by absolute quantification method. The standard curve was prepared with known concentration of plant DNA which extracted from symptomless leaf samples. 50 ng/µl DNA extracted from leaf of virus free okra were tenfold serially diluted up to the final concentration 0.005 ng/µl. For each reaction 20 µl reaction mix was prepared by adding 10 µL SYBR Green mix (PCR BIOSYSTEM, UK), 1.5 µl of plant primer (5 µM) ACT2, 1 µl template DNA and rest of the volume was adjusted with nuclease free water. Quantity of *ACT2* gene in each DNA test sample (extracted using different protocols) was measured in the same reaction plate (96 well PCR plate) by adding 1µl of each DNA samples. No template control (NTC) reactions were carried out with nuclease-free water instead of DNA samples. The qPCR reaction was carried out in duplicates under conditions optimized for the primer (95 °C for 10 min and 40 cycles of 60 °C for 1 min, 95 °C for 15s) using a Step One Plus Real Time PCR system (Applied Biosystems, USA). At the end of the reaction a melting curve analysis was performed from 60 to 95 °C, with an increment of 0.5 °C at 10 s intervals.

E. Detection of virus DNA in extracted total DNA using end point PCR

The extracted total DNA was used as template in PCR for amplification of a segment of begomovirus DNA-A and whole genome of betasatellite associated with OYVMD. Primer pair MKBEGF4 and MKBEGR5 (Venkataravanappa *et al.*, 2012) was used to amplify 1.3 kilobase partial DNA-A and the universal primer pair β01 and β02 (Briddon *et al.*, 2002) were used to amplify the betasatellite DNA (Table 2).

Table 2: List of primers used in end point PCR and qPCR reactions

Primer	Target DNA	Primer sequence (5' to 3')
β01	Beta satellite DNA	GGTACCACTACGCTACGCAG CAGCC
β02		GGTACCTACCCTCCCAGGGG TACAC
MKBEGF4	Segment of DNA-A of Begomovirus	ATATCTGCAGGGNAARATHT GGATGGA
MKBEGR5		TGGACTGCAGACNGGNAAR ACNATGTGGGC
ACT2 F	Segment of actin gene of plant	CTTGCACCAAGCAGCATGAA
ACT2 R		CCGATCCAGACACTGTACTT CCTT

A 20 µl of PCR reaction mix was prepared by adding, 10 µl of PCR readymade mix (PCR BIOSYSTEM, UK), 1 µl of each primer (10 µM), 3 µl of DNA template (10 ng/µl) and 5 µl of nuclease free water. The reactions were carried out in a thermal cycler (Veriti®, Applied Biosystem, USA). For the partial amplification of DNA-A, the cycling conditions were maintained as follows; an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing temperature at 60 °C for 45 s and extension at 72 °C for 1 min with a final extension for 5 min at 72 °C. Similarly, the full length of betasatellite DNA was amplified with following PCR conditions; with initial denaturation at 94 °C for 5 min followed by 35 amplification cycles with melting at 94 °C for 1 min, annealing temperature at 58 °C for 1 min, extension for 1.5 min at 72 °C and final extension at 72 °C for 5 min. For both reactions, the optimum annealing temperature was determined by carrying out the temperature gradient PCR reaction with temperature ranging from 52 °C to 62 °C.

The PCR products were mixed with loading dye and run on 1.5% agarose gel at 90 volts for 30 min. Hyper-ladder I (Bioline, United Kingdom) was used as standard. The gel was stained with ethidium bromide and viewed on a Gel documentation system (UVP ChemiDoc-It TS2 Imager, United Kingdom).

F. Data analysis

Absolute quantification of plant DNA was determined by running the default settings of the StepOne™ Real-Time PCR Software Version 2.3 (Life technologies, USA) on the StepOne Plus™ Real-Time PCR systems (Applied Biosystems USA). The data were subjected to ANOVA in IBM SPSS Statistics for Windows, Version 21.0.

III. RESULTS

Five different DNA extraction protocols were tested to find out the protocol which can yield good quality and quantities of DNA extracted from okra leaves affected by OYVMD. The protocol 4 (modified method I), one of the methods that we introduced in this study, yielded higher concentration of DNA with good quality (Table 3); the average concentration of extracted total DNA was 85.8 ng/µl and the ratios A260/A280 and A260/A230 were 1.8 and 2.1 respectively. The total DNA extracted from 100 mg of the sample was 32.1 µg.

All other extraction methods, except protocol 5 (modified method II), produced a moderate yield; the concentration of the extracted total DNA ranged from 31 ng/µl to 50 ng/µl. However, the nano-drop reading for the quality measure of those DNA samples was not in the acceptable range in most of the samples. The protocol 5 (modified method II) yielded comparatively lowest quantity and quality DNA than other methods tested in this study (Table 3).

Table 3: Nano-drop spectrophotometer reading for the quantity and quality of total DNA extracted from leaf samples of okra

Methods	Concentration (ng/µl)	Total yield (µg/100 mg)[b]	Ratio (A260/A280)[c]	Ratio (A260/A230)[d]
Protocol 1: CTAB method	39.2 ± 2.4a	14.7 ± 1.3	1.6 ± 0.09	1.6 ± 0.02
Protocol 2: An improved method for mucilaginous plant	49.1 ± 2.9	18.4 ± 1.5	1.8 ± 0.07	1.5 ± 0.07
Protocol 3: DNeasy plant mini kit	31.3 ± 1.5	11.7 ± 0.8	1.6 ± 0.07	2.1± 0.12
Protocol 4: Modified method I	85.8 ± 2.1	32.1 ± 1.1	1.8 ± 0.05	2.1± 0.17
Protocol 5: Modified method II	16.3 ± 2.0	6.1 ± 1.0	1.7 ± 0.08	2.4 ± 0.10

[a] Mean value of three biological replicates with standard deviation from mean
[b] Amount of DNA yield in 100 mg leaf powder
[c] Acceptable reading is 1.8
[d] Acceptable range is 2-2.2.

qPCR assay for the quantification of plant DNA in relation to the quantity of actin gene showed results similar to nano-drop quantification in most of the samples (Figure 1). The quantity of the plant DNA in 1 µl of total DNA extracted according to the protocol 4 was 15.45 ng. It is significantly greater than the quantity of plant DNA in the DNA extracts of rest of the protocols. In protocol 2 and protocol 1 the amount of plant DNA was 8.30 ng and 5.81 ng respectively in 1 µl of total DNA samples. The nano-drop reading of DNA quantity for protocol 3 and protocol 5 contradict from qPCR quantification. The quantity of DNA obtained in protocol 3 was twofold higher than obtained in protocol 5. But in qPCR no significant variation was observed. However, in both type of quantifications the amount of DNA in protocol 3 and protocol 5 was comparatively lower than the rest of the protocols.

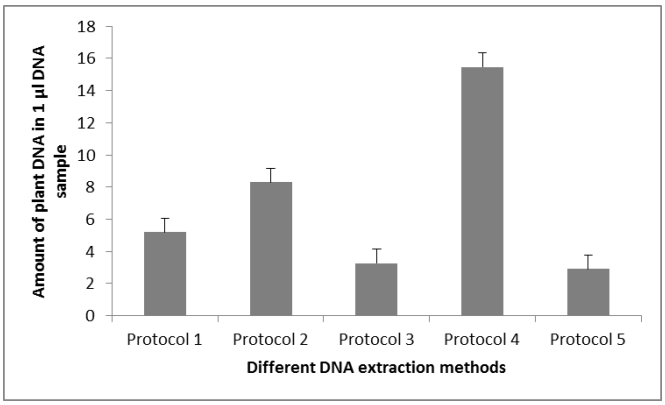

Figure 1. Amount of plant DNA in relation to the amount of actin gene copies in 1 µl total DNA extracted by five different methods. Protocol 1- CTAB method, Protocol 2- An improved method of DNA isolation for mucilaginous plant, Protocol 3- DNeasy plant mini kit, Protocol 4- Modified method I, Protocol 5- Modified method II. S. E. = 0.87.

End-point PCR was carried out to detect the beta-satellite DNA and DNA-A of OYVMV in the extracted total DNA samples. Presence of DNA bands with expected size (about 1.3 kilobase pair) in gel image indicates that all the tested extracts contain virus DNA (Figure 2). However, the intensity of the bands was not similar in all tested samples, which show variation in quantity of DNA in the tested samples. The bright band indicates higher copy number of the virus DNA and faint band indicates lower copy number of the virus DNA. Bright bands were observed in DNA samples extracted using protocol 1, protocol 2 and protocol 4 for both beta-satellite and DNA-A of OYVMV and less faint bands in DNA samples extracted using protocol 5. However, the results obtained for the DNA extracted in protocol 3 was not consistent; a brighter band was noticed for the amplification of DNA-A, but less bright band was for the beta-satellite DNA.

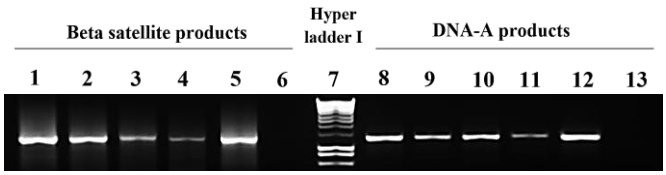

Figure 2. Comparison of different DNA isolation methods based on PCR amplified products of beta-satellite (1-6) and partial DNA-A (8-13). Amplification was detected in all samples with beta-satellite specific primers: (1) modified method I, (2) CTAB method. (3) DNeasy plant mini kit, (4) modified method II, (5) an improved method of DNA isolation for mucilaginous plant. Amplification was detected in all samples with DNA-A specific primers: (8) modified method I, (9) CTAB method. (10) DNeasy plant mini kit, (11) modified method II, (12) an improved method of DNA isolation for mucilaginous plant. No amplification was noticed in non-target control (6, 13).

IV. DISCUSSION

Most of the Malvaceae family members, including okra, have mucilaginous substances in their leaves and other plant parts. The mucilaginous substances greatly interfere in DNA extraction from these plant parts. In the present study, five different DNA extraction methods were tested and analysed to find out a best DNA extraction method that can yield higher quality and large quantity of DNA from okra leaves with the major aim to detect virus DNA. A modified DNA extraction method which was developed in this study (Protocol 4 - Modified method I) produced better quality and quantity of DNA, in addition, the extracted total DNA had large quantities of virus DNA.

Earlier, Ghosh *et al.* (2009) developed a modified CTAB method and recommended for the DNA extraction from highly

mucilaginous plants including okra. However, the protocol has some negative aspects such as using a highly toxic phenol in the extraction, the volume of reagents or buffers are relatively high and it takes too long and hard to re-suspend the DNA pallet in NaCl. In the present study, the modified method 1 is a modified version of the method described by Ghosh *et al.* (2009). However, the quantity of the DNA extracted in modified method 1 is twofold higher than the quantity of DNA extracted in the method described by Ghosh *et al.* (2009) (Table 3). The quality of the DNA extracted using the method described by Ghosh *et al.* (2009) was comparatively lower than the quality of DNA extracted in modified method 1; this might be due to the contamination of some amount of proteins and great amount of carbohydrates and phenolic compounds (Abdel-Latif and Osman., 2017).

Addition of PVP enhances the removal of secondary plant metabolites, especially polyphenolic compounds. The PVP binds with phenolic compounds and helps in their removal (Rezadoost *et al*., 2016). The superfluous quantities of cellular proteins were managed by twice extended treatment with chloroform-isoamyl alcohol. In addition to the removal of proteins, this treatment also helps to remove different coloring substances such as chlorophyll, pigments, and dyes (Azmat *et al*., 2012).

Phenol: chloroform extraction was not included in modified method 1 to avoid potential health hazards. Phenol is irritating to the skin, rapidly absorbed through the skin. Special protections are needed to handle this chemical. Therefore, nowadays researchers try to avoid using phenol in DNA extraction protocols.

In the method proposed by Ghosh *et al*. (2009), the crude nucleic acid pellet was dissolved in 1M NaCl. It took more than 30 min to partially re-suspend the pellet, therefore, it is time consuming and tedious when handling several samples at a time. In modified method 1, TE buffer was used to dissolve crude nucleic acid pellet; the pellet dissolved quickly, within 3 to 5 min. This modification did not affect the quantity and quality of the extracted DNA.

Mostly researchers use RNase to remove RNA that co-extracted with DNA. Studies have proved that RNase removing substantial quantities of DNA under common experimental conditions (Dona and Houseley, 2014). Therefore, prolonged treatment with RNase may also affect the quality and quantity of the DNA. Therefore, the RNase incubation time was reduced to 10 minutes. Dona and Houseley (2014) has proved that RNase treatment followed by phenol:chloroform purification step, as described in Ghosh *et al*. (2009), significantly reduced the yield of the DNA extraction.

Even though both CTAB method (Doyle and Doyle, 1987) and DNeasy plant mini kit method produced a moderated quantity of total DNA, the ratio of A260/A230 or A260/280 was greatly low. This could be due to the contamination of carbohydrates and phenolic compounds. In order to reduce these contaminants, in modified method 1 chloroform-isoamyl alcohol extraction was carried out twice.

The detergent CTAB can be used to isolate highly polymerized DNA from plant. It produces the conformational change in the DNA from random coil to compact globule, making DNA precipitation more effective. CTAB and chloroform:isoamyl alcohol mixture separates contaminants into the organic phase and nucleic acid into the aqueous phase (Doyle and Doyle, 1987). But, many plants have very high levels of secondary metabolites, including lipids, phenolic compounds and viscous polysaccharides. These can be removed by doing further processing, commonly with organic solvents, such as phenol or other toxic compounds (Pedersen *et al.*, 2006; Bashalkhanov and Rajora, 2008; Bellstedt *et al.*, 2010). The phenolic compounds are powerful oxidizing agents and bind covalently to extracted DNA making it useless for most of the molecular manipulation. Thus, if they are not removed properly, then they hinder subsequent downstream assays including PCR (Porebski *et al*., 1997; Angeles *et al.*, 2005).

Using commercial DNA extraction kits is easier, faster and relatively less hazardous than the conventional extraction methods. The extraction using commercially available kits consist of comparatively few steps for the completion of the entire extraction process, but it is expensive. For experiments with large scale DNA extraction and screening, the cost of the kits gives extra burden, especially to the researchers in developing countries. Conversely, conventional methods are cheaper even though they consume too long for extraction steps. The average completing time for the modified method1 was approximately 2 ½ hours, handling 10 samples at the same time. In overall, the results indicated that the DNA extraction using modified method 1 is advantageous over the other conventional methods with respect to the time, yield of the DNA and reproducibility by PCR.

There have been numerous reports of extraction of begomoviral DNA from wide range of plant species rich in mucilage and polysaccharides including okra (Jose and Usha, 2000), Bixa (Echevarria-Machado *et al.*, 2005), Sedum (Barnwell *et al.*, 1998), cotton (Mansoor *et al.*, 1999), forest plants (Scott and Playford, 1996) and legumes (Rouhibakhsh *et al*., 2008). Besides their specificity towards a particular plant species, most of these protocols are either time consuming or involve some additional reagents such as polyethylene glycol and silica which make these methods expensive and cumbersome.

Based on the findings of this study, the modified method 1 is a highly suitable method for the extraction of DNA from okra, because of higher yield and good quality of the extracted DNA. The DNA extracted in this method is highly useful in

downstream applications such as PCR based detection, identification and quantification of virus DNA.

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VI. REFERENCES

Abdel-Latif, A and Osman, G, (2017). Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods* 13:1. DOI 10.1186/s13007 016 0152-4

Angeles, J, Laurena, A and Evelyn Mae T, (2005). Extraction of Genomic DNA From the lipid- ,polysaccharide-, and olyphenol-rich cocont (*Cocos nucifera* L.). *Plant Molecular Biology Reporter* 23, 297–298.

Axel Diederichsen, J, Philip, R and Duguid S, (2006). Variation of mucilage in flax seed and its relationship with other seed characters. *Crop Science* 46, 365– 371.

Azmat, M. A, Khan, I. A, Cheema, H. M. N, Rajwana, I. A, Khan, A. S and Khan, A. A, (2012). Extraction of DNA suitable for PCR applications from mature leaves of *Mangifera indica* L. *Journal of Zhejiang University Science B* 13, 239-243.

Barnwell, P, Blanchard, A, Bryant, J, Smirnoff, N and Weir, A, (1998). Isolation of DNA from the highly mucilagenous succulent plant shape *Sedum telephium*. *Plant Molecular Biology Reporter* 16, 133.

Bashalkhanov, S and Rajora, O, (2008). Protocol: a high-throughput DNA extraction system suitable for conifers. *Plant Methods* 4, 20.

Bayer, C, Fay, M and De Bruijn, A, (1999). Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: a combined analysis of plastid atpB and rbcL DNA sequence. *Botanical Journal of the Linnean Society* 129, 267–303.

Bellstedt, D, Pirie, M, Visser, J, DeVilliers, M and Gehrke, B, (2010). A rapid and inexpensive method for the direct PCR amplification of DNA from plants. *American Journal of Botany* 97, 65–68.

Briddon, R. W, Bull, S. E, Mansoor, S, Amin, I and Markham, P. G, (2002). Universal primers for the PCR-mediated amplification of DNA β. *Molecular Biotechnology* 20, 315–318.

Briddon, R and Markham, P, (1994). Universal primers for the PCR amplification of dicot-infecting geminiviruses. *Molecular Biotechnology* 1, 202–205.

Cavallari, M. M, Siqueira, M. V. B. M, Val, T. M and Pavanelli, J. C, (2014). A modified acidic approach for DNA extraction from plant species containing high levels of secondary metabolites. *Genetics and Molecular Research* 13, 6497–6502.

Che, P, Bussell, J. D, Zhou, W, Estavillo, G. M, Pogson, B. J and Smith, S. M, (2010). Signalling from the endoplasmic reticulum activates brassinosteroid signalling and promotes acclimation to stress in *Arabidopsis*. *Science Signaling* 3, ra69.

Dellaporta, S, Wood, J and Hicks, J, (1983). A plant DNA mini-preparation Version II. *Plant Molecular Biology Reporter* 1, 19–21.

Deng, D, Mcgrath, P. F and Harrison, B. D, (1994). Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Annals of Applied Biology* 125, 327–336.

Dona, F and Houseley, J, (2014) Unexpected DNA loss mediated by the DNA binding activity of ribonuclease A. PLoS *ONE* 9(12): e115008. doi:10.1371/journal.pone.0115008

Doyle, J and Doyle, J, (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin* 19, 11–15.

Echevarria-Machado, I, Lucila, A, Sanchez, C, Hernandez-Zepeda, C, Rivera-Madrid, R and Moreno-velenzuela O, (2005). A simple and efficient method for isolation of DNA in high mucilaginous plant tissues. *Molecular Biotechnology* 31, 129–135.

Fang, G, Hammer, S and Grumet, R, (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Bio Techniques* 13, 52–56.

Ghosh, R, Paul, S and Das, S, (2008). Molecular evidence for existence of a New World begomovirus associated with yellow mosaic disease of *Corchorus capsularis* in India. *Australasian Plant Disease Notes* 3, 59–62.

Ghosh, R, Paul, S, Ghosh, S. K and Roy A, (2009). An improved method of DNA isolation suitable for PCR-based detection of begomoviruses from jute and other mucilaginous plants. *Journal of Virological Methods* 159, 34–39.

Höfer, P, Engel, M, Jeske, H and Frischmuth, T, (1997). Host range limitation of a pseudo recombinant virus produced by two distinct bipartite geminiviruses. *Molecular Plant-Microbe Interactions* 10, 1019–1022.

Jose, J and Usha, R, (2000). Extraction of Geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*). *Plant Molecular Biology Reporter* 18, 349–355.

Jose, J and Usha, R, (2003). Bhendi Yellow Vein Mosaic Disease in India Is Caused by Association of a DNA betasatellite with a Begomovirus. Virology 317, 310–317.

Kawamura, T, Hisata, Y and Okuda K, (2000). Diversity of alkaloid and mucilage in phellodendron barks from different habitats. *Nature Medicine* 54, 193–195.

Khan, A.J, Al-saady, N.A, Al-mahruki, M.S, Al-oufi, M and Al-subhi A.M, (2007). Molecular characterization of Begomovirus infecting sweet pepper in Oman. *Indian Journal of Biotechnology* 6, 45–51.

Kushwaha, N, Singh, A.K, Chattopadhyay, B and Chakraborty, S, (2010). Recent advances in geminivirus detection and future perspectives. *The Journal of Plant Protection Sciences* 2, 1–18.

Mansoor, S, Bashir, A and Khan, S, (1999). Rapid multiplex PCR for the specific detection of two whitefly-transmitted geminivirus species associated with cotton leaf curl disease in Pakistan. *Pakistan Journal of Botany* 31, 115–123.

Muralikrishna, G, Bhat, U and Tharanathan, R, (1989). Functional characteristics of the mucilaginous polysaccharides derived from cowpea (*Vigna sinensis*), black gram (*Phaseolus mungo*) and linseed (*Linum usitatissimum*). *Starch* 39, 107–109.

Pedersen, N, Russel, S, Newton, A and Ansell, S, (2006). A novel molecular protocol for the rapid extraction of DNA from byrophytes and the utility of direct amplification of DNA from a single dwarf male. *The Bryologist* 109, 257–264.

Porebski, S, Bailey, L and Banum, B, (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter* 15, 8–15.

Rezadoost, M.H, Kordrostami, M and Kumleh, H.H, (2016). An efficient protocol for isolation of inhibitor-free nucleic acids even from recalcitrant plants. *3 Biotech* 6, 61.

Rothenstein, D, Haible, S, Dasgupta, I, Dutt, N, Patil, B and Jeske, H, (2005). Biodiversity and recombination of cassava-infecting begomoviruses from southern India. *Archives of Virology* 151, 55–69.

Rouhibakhsh, A, Priya, J, Periasamy, M, Haq, Q and Malathi, V, (2008). An improved DNA isolation method and PCR protocol for efficient detection of multicomponents of begomovirus in legumes. *Journal of Virological Methods* 147, 37–42.

Roy, A, Acharyya, S and Das, S, (2009). Distribution, epidemiology and molecular variability of the begomovirus complexes associated with yellow vein mosaic disease of mesta in India. *Virus Research* 141, 237–246.

Scott, K and Playford, J, (1996). DNA extraction technique for PCR in rain forest plant species. *Bio Techniques* 20, 974–978.

Sharma, P, Rishi, N and Malathi V.G, (2005). Molecular cloning of coat protein gene of an Indian cotton leaf curl virus (CLCuV-HS2) isolate and its phylogenetic relationship with others members of geminiviridae. *Virus Genes* 30, 85–91.

Swanson, M, Varma, A, Muniyappa, V and Harrison, B, (1992). Comparative epitope profiles of the particle proteins of whitefly-transmitted geminiviruses from nine crop legumes in India. *Annals of Applied Biology* 120, 425–433.

Venkataravanappa, V, Reddy, C.N.L, Jalali, S and Reddy M. K, (2012). Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes* 44, 522–535.

Diversity and Abundance of Waterbird Communities in the Jaffna and Kilinochchi Districts: Where do we have to go from here?

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Abstract— Wetlands and waterbirds are inseparable elements. The preliminary study was conducted in eight sites, namely Mandaitivu, Mankumban, Kayts, Kavutharimunai, Pallai, Thadduvankoddy, Kapputhu and Nagarkovil in the Jaffna and Kilinochchi Districts of the northern region of Sri Lanka to determine the diversity and abundance of waterbirds. In each site, three counting blocks in length of 500 m with open width was selected for bird counting. Each block was separated at least by length of 500 m to avoid double counting. The counting of birds was done once a month from December, 2016 to May, 2017. Eighteen waterbird families were recorded. Phoenicopteridae, Anatidae and Scolopacidae were the dominant families. The presence of critically endangered species such as Spot-billed Duck (*Anas poecilorhyncha*) suggests the importance of conservation of these habitats. Two-way ANOVA indicated that waterbird families were significantly different among eight sites. The highest species richness was found in Kapputhu (57) and the lowest in Kavutharimunai (26). This study revealed that Mandaitivu, Mankumban, Kayts, Thadduvankoddy and Kapputhu are the excellent places to observe both migrant ducks and flamingos. Likewise Kavutharimunai is good for migrant shorebirds and seabirds by ferry while Nagarkovil and Kapputhu are suitable for viewing both feeding and roosting of waterbirds. On the whole this paper reveals that the northern region of Sri Lanka possess not only the potential of avitourism but also will become the hotspot of future bird researches, especially on waterbirds.

Keywords— Jaffna, Kilinochchi, waterbird communities, avitourism, hotspot

I. INTRODUCTION

Wetlands and waterbirds are inseparable elements (Grimmett and Inskipp, 2007). According to Ramsar convention, waterbirds have been defined as species of birds that are ecologically dependent upon wetlands (Wetlands International, 2010). Waterbirds inhabit or temporarily use wetlands because of the diversity of microhabitats for feeding, nesting, resting and roosting (Hattori and Mae, 2001). At present, 454 species of birds including 238 breeding resident and 144 regular migrants and 72 vagrants were recorded in Sri Lanka. 23 families of waterbirds (164 species) have been identified in Sri Lanka (Wijesundara *et al*., 2017).

Wetlands in Sri Lanka are the final destination each year for tens of thousands of migrant birds flying southward along the Central- Asian 'flyway' (Warakagoda and Sirivardana, 2006). Migratory birds that use the Central Asian flyway, use five main entry and exit points to enter Sri Lanka, of which three are located in the Northern Province of Sri Lanka namely, the Jaffna Peninsula, the Adam's Bridge/Mannar region and the Devil's Point (Kotagama and Ratnavira, 2010).

Although bird-related studies have been undertaken in the northern region of Sri Lanka (Kandasamy *et al.,* 2016; Kandasamy *et al.,* 2017a; Kandasamy *et al.,* 2017b; Kandasamy *et al.,* 2017c; Kandasamy *et al.,* 2017d; Kandasamy *et al.,* 2018; Rajkumar and Wijesundara, 2014; Wijesundara *et al.,* 2015; Wijesundara *et al.,* 2017; Rajkumar and Wijesundara, 2015a; Rajkumar and Wijesundara, 2015b; Wijesundara *et al*., 2015; Rajkumar and Wijesundara, 2017; Wijesundara and Rajkumar, 2016; Wijesundara *et al.,* 2016; Wijesundara and Rajkumar, 2017; Wijesundara *et al.,* 2018) waterbird communities have not been studied in many areas in the northern region of Sri Lanka as the area was inaccessible for three decades due to the armed conflict. Hence the present study was conducted to document the diversity and abundance of waterbirds in these districts and to find out the possibilities of ecotourism in selected areas in this region.

II. MATERIALS AND METHODS

The present study was carried out in eight locations (Figure 1) namely, Mandaitivu in the Island South Divisional secretariat division (DSD (9^0 37' 28" N, 79^0 59' 52" E), Mankumban in the Island South DSD (9^0 38' 31" N, 79^0 56' 28" E), Kayts in the Island North DSD (9^0 40' 17" N, 79^0 55' 30" E), Kavutharimunai in the Pooneryn DSD (9^0 34' 42" N, 80^0 06' 24" E), Pallai wind farm in the Pachchilappalli DSD (9^0 35' 59" N, 80^0 18' 60" E), Thadduvankoddy in the Kandavalai DSD (9^0 30' 0" N, 80^0 25' 0" E), Kapputhu in the Vadamaradchi South West DSD (9^0 44' 09" N, 80^0 10' 48" E), and Nagarkovil in the Vadamaradchi East DSD (9^0 36' 00" N, 80^0 17' 00" E).

A line transect was established in each study site, the entire transect belt was divided into three blocks in length of 500 m with open width. Each block was separated at least by 500 m

to avoid double counting as most were open areas. All the waterbirds seen on either side of the transect up to 500 m were counted during dawn and noon or dusk on alternate months to capture temporal variations by walking along the transect line of each block. Waterbird counting was done by ferry in Kavutharimunai with the help of the Fisheries' Society, Maniththalai, Kavutharimunai. Waterbird species were identified using a standard field guide (Harrison, 2011). Counting blocks were visited once a month from December, 2016 to May, 2017. Then a list of status of waterbirds was prepared following Harrison, 2011 and Wijesundara *et al*., 2017.

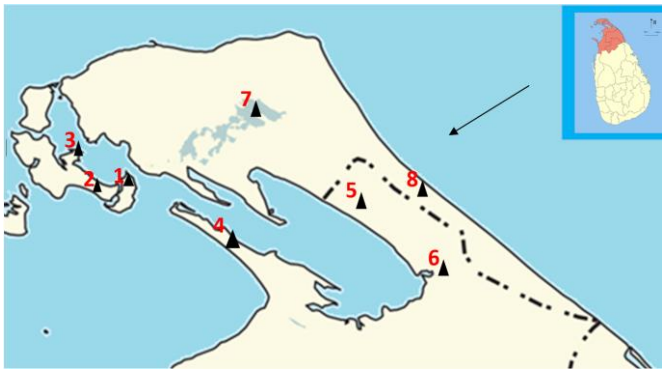

Figure 1: The eight study sites denoted with numbers. 1: Mandaitiv, 2: Mankumban, 3: Kayts, 4: Kavutharimunai, 5: Pallai, 6: Thadduvankoddy, 7: Kapputhu and 8: Nagarkovil.

III. Data Analysis

Diversity indices such as Shannon Diversity Index (H), Pielou Index (J), Margalef's Richness Index and Berger-Parker Dominance Index were used to compare the diversity and abundance of waterbirds in eight study sites (Magurran, 2004). The percentage of occurrence was compared for families of waterbirds recorded. Abundance of waterbird families were compared by creating the dendrogram with the help of Minitab 17 to find out similarities among the study sites. Two-way ANOVA was also calculated by using SPSS 14 to find out the variations in the diversity and abundance of birds with regards to the study sites.

IV. Results and Discussion

Current study recorded a total of 65 waterbird species belonging to 18 families (Figure 2). Of these, 27 species were migrants, 1 was mainly migrant with resident population, another 5 were mainly residents with migrant population, 2 were mainly residents with doubtful migrant status, and rest of the 30 were residents. Among them, 12 waterbird species were recorded in all eight sites (Table 3). The highest species richness was found in Kapputhu (57) and the lowest in Kavutharimunai (26). 116 species of waterbirds belonging to 23 families were recorded in the Jaffna, Kilinochchi, Mullaitivu and in Mannar in the northern region of Sri Lanka in a previous study (Wijesundara *et al.*, 2017).

A. Species richness, diversity and evenness

Among the eight locations, the highest measure of species richness was found in Kapputhu (57) (Table 1). However, the Shannon-Wiener diversity index was comparatively lower (2.92) in Kapputhu compared to Mandaitivu (3.22) due to the domination of two families Phoenicopteridae (percentage of occurrence 26.46%) and Anatidae (20.96%) in Kapputhu. Berger Parker dominance index was higher in Kapputhu (0.26) compared to Mandaitivu (0.16).

The highest Shannon diversity (3.22) and even distribution (0.84) and the lowest Berger Parker dominance (0.16) were found in the waterbird assemblages in Mandaitivu. This may be due to the presence of rich microhabitats such as mangroves, marshlands, open water pools, wet and dry mudflats that provide habitats for a range of waterbirds. As far as Mandaitivu, Mankumban and Kayts were concerned, migrant ducks (Family Anatidae), showed high abundance (38.46%, 37.03% and 38.09% respectively).

The lowest species richness and the lowest Shannon diversity and evenness were found in Kavutharimunai. The intertidal area and shallow waters of this location dominated by a large congregation of shorebirds belong to family Scolopacidae (54.79%) and seabirds of families, Sternidae (15.58%) and Laridae (11.12%) for feeding and resting which may have resulted the lowest species richness and diversity measures in Kavutharimunai.

Based on the abundance, Phoenicopteridae and Anatidae were the highly dominated waterbird families in Thadduvankoddy (Figure 02). Families Scolopacidae and Phalacrocoracidae were the dominant families in the seashore near the Pallai wind farm and Nagarkovil respectively. The deep water (>1 m) and dense mangrove vegetation of Nagarkovil provided suitable feeding and roosting for diving birds of Family Phalacrocoracidae. As such these observations showed the ecological value of these areas in the development of avitourism in the northern region of Sri Lanka.

A significant site effect (Table 2) indicated that waterbird families were different among the eight locations. Habitat heterogeneity, availability of water and food and disturbances

Table 1: Comparison of species richness, diversity, evenness, richness and dominance indices in eight study sites.

Variable	1	2	3	4	5	6	7	8
Species Richness	47	49	41	26	37	50	57	41
Shannon – Wiener (H)	3.22	2.89	2.71	1.92	2.57	2.46	2.92	2.57
Pielou Index (J)	0.84	0.74	0.73	0.58	0.71	0.62	0.72	0.68
Margalef's Species Richness index	6.50	5.56	5.13	3.42	5.48	5.64	6.30	5.36
Berger Parker Dominance Index	0.16	0.21	0.27	0.53	0.35	0.32	0.26	0.27

1: Mandaitiv, 2: Mankumban, 3: Kayts, 4: Kavutharimunai, 5: Pallai, 6: Thadduvankoddy, 7: Kapputhu and 8: Nagarkovil.

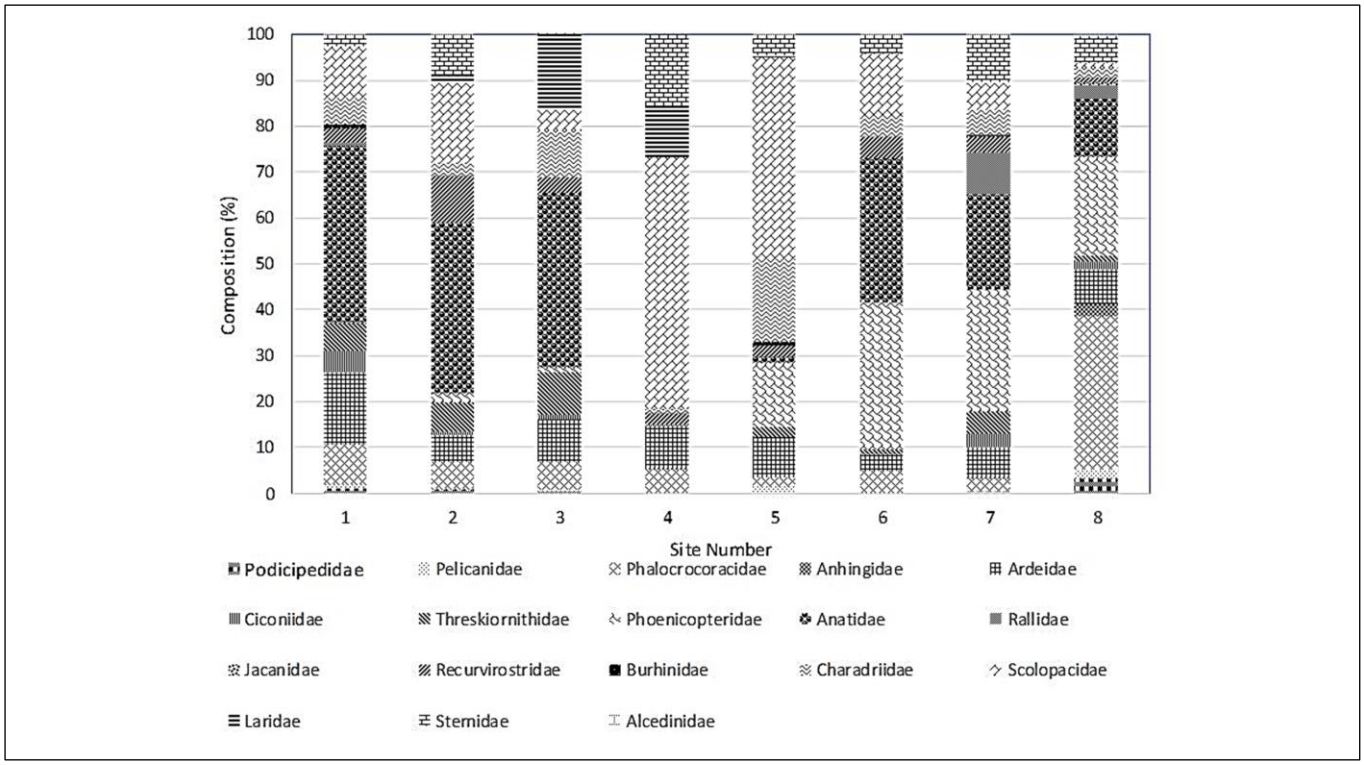

Figure 2: Composition of waterbird families (%) in eight locations; 1: Mandaitivu, 2: Mankumban, 3: Kayts, 4: Kavutharimunai, 5: Pallai, 6: Thadduvankoddy, 7: Kapputhu and 8: Nagarkovil.

may cause an effect in spatial variation in waterbird families in eight locations.

The dendrogram (Figure 3) showed three major clusters. The study sites namely Mandaitivu (1), Pallai (5), Kavutharimunai (4), Kayts (3), Nagarkovil (8) were closely related in terms of abundance of waterbirds compared to Mankumban (2), Thadduvankoddy (6) and Kapputhu (7). Kavutharimunai (Site 4) and Pallai (5) were clustered together because both were dominated by Scolopacidae. Mankumban (2), Thadduvankoddy (6) were clustered together. This may be due to the similarity in percentage in the most dominant waterbird family, Anatidae. Kapputhu (7) was separated from other sites. The highest species richness and high Shannon diversity Index next to Mandaitivu were recorded in Kapputhu. Therefore Kapputhu was found to be the most suitable area amongst all the sites to observe numerically abundant waterbird species with high species richness.

Table 2: The result of the two-way ANOVA (Tests of Between-Subject Effects) for the two variables (month and site) and their interaction. (Statistically significance levels in terms of abundance of waterbird species are given)

Source	Degrees of freedom	F	Significance (<0.05)
Corrected Model	47	1.66	0.02
Intercept	1	82.56	0
Month	5	1.11	0.36
Site	7	6.32	0
Interaction (Month × Site)	35	0.80	0.76

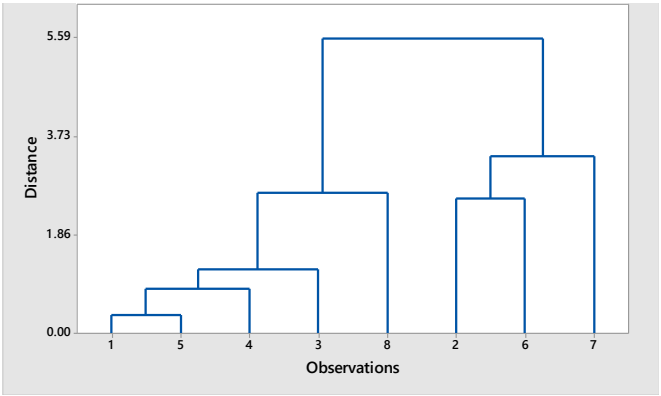

Figure 3: Dendrogram showing the relationship among the study sites based on the abundance of waterbirds (1: Mandaitivu, 2: Mankumban, 3: Kayts, 4: Kavutharimunai, 5: Pallai, 6: Thadduvankoddy, 7: Kapputhu and 8: Nagarkovil)

B. Threatened species in Sri Lanka

Spot-billed Duck (*Anas poecilorhyncha*) were recorded in Mandaitivu, Mankumban, Kayts, and Kapputhu (Figure 4). They were most abundant in Mankumban. They are considered as critically endangered species in Sri Lanka (Weerakoon, and Gunawardena, 2012). Food availability, water level and habitat structure of mangroves and saltmarsh ecosystem in the study area might provide suitable habitat for them, suggesting the importance of conservation of these habitats.

Figure 4: Spot-billed duck (*Anas poecilorhyncha*) in Kayts (Photograph by Gajavathany Kandasamy).

V. CONCLUSION

Mandaitivu, Mankumban, Kayts, Thadduvankoddy, Kapputhu and Nagarkovil were excellent habitats to observe waterbird families of Anatidae and Phoenicopteridae. Kavutharimunai is suitable to observe families Scolopacidae, Laridae and sternidae by ferry. These are potential areas for avitourism in the northern region of Sri Lanka. Moreover, Kapputhu and Nagarkovil are suitable to observe both feeding and roosting of waterbirds. The current study recognizes these sites as important habitats for protection of waterbirds and for the development of avitourism in the northern region.

VI. FUTURE DIRECTIONS AND RESEARCH PRIORITIES

Avitourism is an emerging sub-sector of the nature-based tourism industry (Steven *et al.,* 2015). Bird related studies were rare in the northern region of Sri Lanka nearly for three decades due to the armed conflict. In recent times most of the published researches focused on diversity and abundance of waterbirds in the northern region, Sri Lanka. Studies have identified considerable number of birding areas with a high avitourism potential in the northern region of Sri Lanka (Kandasamy *et al.,* 2016; Kandasamy *et al,* 2017a; Kandasamy *et al,* 2017b; Kandasamy *et al,* 2017c; Kandasamy *et al,* 2017d; Kandasamy *et al,* 2018; Rajkumar and Wijesundara, 2014; Wijesundara *et al.,* 2015; Wijesundara *et al.,* 2017; Rajkumar and Wijesundara, 2015a; Rajkumar and Wijesundara, 2015b; Wijesundara *et al.,* 2015; Rajkumar and Wijesundara, 2017; Wijesundara and Rajkumar, 2016; Wijesundara *et al.,* 2016; Wijesundara and Rajkumar, 2017; Wijesundara *et al.,* 2018).

In addition to identify the potential locations in the northern region of Sri Lanka, collaborations are needed between the natural and social science community to investigate the attitudes of currently visiting avitourists and the local public towards birds, bird habitats and bird conservation for the development of sustainable avitourism in the northern region of Sri Lanka. A more detailed research is needed to assess specific bird groups and locations in the northern region, Sri Lanka that can attract avitourists. This kind of research will direct to find opportunities for the development of avitourism and to find which sites may be vulnerable to the potential negative impacts of the industry.

The ecology of waterbirds is poorly known in the northern region of Sri Lanka. In the present study 18 waterbird families were recorded in the region. Each family has different foraging strategies for their survival. During migratory seasons, foraging is the most important activity for shorebirds, as it allows them to survive and ensures their safe arrival at the breeding ground (Norazlimi and Ramli, 2015). Moreover, different locations rich in different microhabitats support various waterbird families. A good knowledge of their habitat requirements is essential for the management and conservation of wetlands for the protection of waterbirds.

Foraging strategies play important roles in the habitat use of shorebirds at stopover sites and wintering grounds. Shorebirds with different foraging strategies select different habitats and use different food resources, and this may effectively avoid interspecific competition (Jing *et al.,* 2007). As such studies on abundance and availability of food in each habitat and habitat usage patterns of waterbirds are important in these areas. Therefore studies on feeding ecology and habitat use remain high priorities for the waterbird conservation of the northern region, Sri Lanka.

Table 3: Status of waterbird species in the eight study sites

	Family name	Common Name	Scientific Name	Resident / Migrant	NCS	GCS
1	Podicipedidae	Little Grebe	*Tachybaptus ruficollis*	R	LC	LC
2	Pelecanidae	Spot-billed Pelican	*Pelecanus philippensis*	R	LC	NT
3	Phalacrocoracidae	Indian Cormorant	*Phalacrocorax fuscicollis*	R	LC	LC
4	Phalacrocoracidae	Little Cormorant	*Phalacrocorax niger*	R	LC	LC
5	Anhingidae	Oriental Darter	*Anhinga melanogaster*	Uncommon R	LC	NT
6	Ardeidae	Grey Heron	*Ardea cinerea*	R	LC	LC
7	Ardeidae	Purple Heron	*Ardea purpurea*	R	LC	LC
8	Ardeidae	Great Egret	*Egretta alba*	R	LC	LC
9	Ardeidae	Intermediate Egret	*Ardea intermedia*	R	LC	LC
10	Ardeidae	Little Egret	*Egretta garzetta*	R	LC	LC
11	Ardeidae	Cattle Egret	*Ardea ibis*	R.M?	LC	LC
12	Ardeidae	Indian Pond Heron	*Ardeola grayii*	R	LC	LC
13	Ardeidae	Striated Heron	*Butorides striatus*	R	LC	LC

Table 3: (continued).

	Family name	Common Name	Scientific Name	Resident / Migrant	NCS	GCS
14	Ardeidae	Yellow Bittern	*Ixobrychus sinensis*	R,M	NT	LC
15	Ciconiidae	Painted Stork	*Mycteria leucocephala*	R	LC	NT
16	Ciconiidae	Asian Openbill	*Anastomus oscitans*	R	LC	LC
17	Threskiornithidae	Black-headed Ibis	*Threskiornis melanocephalus*	R	LC	NT
18	Threskiornithidae	Glossy Ibis	*Plegadis falcinellus*	M		LC
19	Threskiornithidae	Eurasian Spoonbill	*Platalea leucorodia*	R	LC	LC
20	Phoenicopteridae	Greater Flamingo	*Phoenicopterus roseus*	M		LC
21	Anatidae	Lesser Whistling Teal	*Dendrocygna javanica*	R	LC	LC
22	Anatidae	Eurasian Wigeon	*Anas penelope*	M		LC
23	Anatidae	Northern Shoveller	*Anas clypeata*	M		LC
24	Anatidae	Northern Pintail	*Anas acuta*	M		LC
25	Anatidae	Garganey	*Anas querquedula*	M		LC
26	Anatidae	Common Teal	*Anas crecca*	M		LC
27	Anatidae	Spot-billed Duck	*Anas poecilorhyncha*	R,M?	CR	LC
28	Rallidae	White-breasted Waterhen	*Amaurornis phoenicurus*	R	LC	LC
29	Rallidae	Purple Swamphen	*Porphyrio porphyrio*	R	LC	LC
30	Rallidae	Common coot	*Fulica atra*	R	LC	LC
31	Rallidae	Common Moorhen	*Gallinula chloropus*	R	LC	LC
32	Jacanidae	Pheasant-tailed Jacana	*Hydrophasianus chirurgus*	R	LC	LC
33	Recurvirostridae	Black-winged Stilt	*Himantopus himantopus*	R,M	LC	LC
34	Burhinidae	Great Thick-knee	*Esacus recurvirostris*	R	LC	LC
35	Charadriidae	Yellow-wattled Lapwing	*Vanellus malabaricus*	Uncommon R	LC	LC
36	Charadriidae	Red-wattled Lapwing	*Vanellus indicus*	R	LC	LC
37	Charadriidae	Asiatic Golden Plover	*Pluvialis fulva*	M		LC
38	Charadriidae	Common Ringed Plover	*Charadrius hiaticula*	M		LC
39	Charadriidae	Little Ringed Plover	*Charadrius dubius*	R,M	VU	LC
40	Charadriidae	Kentish Plover	*Charadrius alexandrinus*	R,M	VU	LC
41	Charadriidae	Monglian Plover	*Charadrius mongolus*	M		LC
42	Scolopacidae	Black-tailed Godwit	*Limosa limosa*	M		NT
43	Scolopacidae	Whimbrel	*Numenius phaeopus*	M		LC
44	Scolopacidae	Eurasian Curlew	*Numenius arquata*	M		NT
45	Scolopacidae	Common Redshank	*Tringa totanus*	M		LC
46	Scolopacidae	Common Greenshank	*Tringa nebularia*	M		LC
47	Scolopacidae	Green Sandpiper	*Tringa ochropus*	M		LC
48	Scolopacidae	Marsh Sandpiper	*Tringa stagnatilis*	M		LC
49	Scolopacidae	Wood Sandpiper	*Tringa glareola*	M		LC
50	Scolopacidae	Common Sandpiper	*Actitis hypoleucos*	M		LC
51	Scolopacidae	Pintail Snipe	*Gallinago stenura*	M		LC
52	Scolopacidae	Little Stint	*Calidris minuta*	M		LC
53	Scolopacidae	Curlew Sandpiper	*Calidris ferruginea*	M		NT
54	Laridae	Great Black-headed Gull	*Larus ichthyaetus*	M		LC
55	Laridae	Brown-headed Gull	*Larus brunnicephalus*	M		LC
56	Sternidae	Whiskered Tern	*Chlidonias hybridus*	M		LC
57	Sternidae	White-winged Tern	*Chlidonias leucopterus*	M		LC
58	Sternidae	Gull-billed Tern	*Sterna nilotica*	M,R	CR	LC
59	Sternidae	Caspian Tern	*Sterna caspia*	R,M	CR	LC
60	Sternidae	Little Tern	*Sterna albifrons*	R	VU	LC
61	Sternidae	Lesser-Crested Tern	*Sterna bengalensis*	M		LC
62	Sternidae	Great-crested Tern	*Sterna bergii*	R	NT	LC
63	Alcedinidae	Pied Kingfisher	*Ceryle rudis*	R	LC	LC
64	Alcedinidae	Common Kingfisher	*Alcedo atthis*	R	LC	LC
65	Alcedinidae	White-throated Kingfisher	*Halcyon smyrnensis*	R	LC	LC

NCS- National Conservation Status, GCS – Global Conservation Status, CR-Critically Endangered, R - Resident, M – Migrant
M, R denotes the main populations being migrant, with minor resident populations.
R, M denotes the main populations being resident, with minor migrant populations
R, M? denotes species were mainly residents with doubtful migrant status
Status of waterbirds according to Wijesundara et al., (2017).
E- Endangered, LC - Least Concerned, VU- Vulnerable (Weerakoon and Gunawardena, 2012).

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VIII. REFERENCES

Grimmett, R. and Inskipp T. (2007). Edn.2. *Birds of the Indian Subcontinent.* Christopher Helm (Publishers) Ltd., London. 528 pp.

Harrison, J, A. (2011). *A Field Guide to the Birds of Sri Lanka*. Oxford University Press, Oxford. 208 pp.

Hattori, A. and Mae, S. (2001). Habitat use and diversity of waterbirds in a coastal lagoon Lake Biwa, Japan. *Ecological Research* Vol. 16: 543 – 553.

Jing, K., Zhijun, M., Li, B., Li, J., and Chen, J. (2007) Foraging strategies involved in habitat use of shorebirds at the intertidal area of Chongming Dongtan, China. *Ecological Research*, Vol.22: 559–570. DOI 10.1007/s11284-006-0302-7.

Kandasamy G, Wijesundara C, and Sivaruban A. (2016). Comparison of avifaunal diversity in different habitats in the Jaffna Peninsula. Pages 362 *in* Peradeniya University Research Proceedings, 2016: 362.

Kandasamy G., Weerakoon, D. K., Sivaruban, A., and Jayasiri, H. B (2017a). Spatial variation of waterbirds in eight selected sites in the Northern Province of Sri Lanka, *Australasian Ornithology Conference:* 47.

Kandasamy G., Weerakoon, D. K., and Sivaruban, A. (2017b). Spatial variation of waterbirds in Pallai and Thadduvankoddy in the Northern Province of Sri Lanka, *Third International Conference on Science, Engineering and Environment, SEE-USQ, 2017, Brisbane, Australia*: 124-129.

Kandasamy, G., Weerakoon, D. K., Sivaruban, A., and Jayasiri, H. B (2017c). Spatial variation of waterbirds in Mandaitivu and Kavutharimunai in the Northern Province, Sri Lanka. Online page *in* Student Conference of Conservation Science (SCCS).

Kandasamy, G., Weerakoon, D. K., and Sivaruban, A. (2017d). Spatial variation of waterbirds in Kapputhu and Nagarkovil in the Jaffna Peninsula. Forty Years of Bird Research – Field Ornithology Group of Sri Lanka, *FOGSL symposium, Faculty of Science, University of Colombo.*

Kandasamy, G., Weerakoon, D. K., Sivaruban, A., and Jayasiri, H. B (2018). Waterbird diversity in Kavutharimunai in the Northern Province of Sri Lanka, *National Aquatic Resources and Development Agency (NARA) International Scientific Sessions:* 42.

Kotagama, S., Ratnavira, G. (2010). An illustrated guide to the birds of Sri Lanka. Field Ornithology Group of Sri Lanka, Colombo. 382 pp.

Magurran, A. E. (2004) Measuring Biological Diversity. *Blackwell Publishing, Oxford*, 256 pp.

Norazlimi, N, A. and Ramli, R. (2015). The Relationships between morphological characteristics and foraging behavior in four selected species of shorebirds and waterbirds utilizing tropical mudflats. *The Scientific World Journal*, Vol. 2015: 1-7.

Rajkumar P. and Wijesundara C. (2014). Preliminary observations on migratory birds on the Island of Mandaitivu, Jaffna, Sri Lanka. Pages 30-30 *in* Proceedings of the Peradeniya University. International Research Sessions, Sri Lanka, Vol. 18.

Rajkumar, P., and C. Wijesundara. (2015a). Avifaunal diversity of Thondaimanaru Lagoon and adjacent areas, Jaffna, Sri Lanka as an indicator of water quality. Pages 24-24 *in* Fourth International Symposium on Water Quality and Human Health. Postgraduate Institute of Science, University of Peradeniya, Peradeniya.

Rajkumar, P., and C. S. Wijesundara. (2015b). Developing bird-based ecotourism in Jaffna: A case study on mangrove habitats on the islands of Mandaitivu and Sirudivu. Pages 311-311 *in* Peradeniya University International Research Sessions. University of Peradeniya, Peradeniya.

Rajkumar, P., and C. S. Wijesundara. (2017). Diversity and abundance of migratory birds in Sarasalai and Anthanathidal mangroves in Jaffna District, Sri Lanka. Page 8-8 *in* PGIS Research Congress 2017. University of Peradeniya, Sri Lanka, Postgraduate Institute of Science, University of Peradeniya, Sri Lanka.

Steven, R., Morrison, C., Castley, J. G. (2014). Bird watching and avitourism: A global review of research into its participant markets, distribution and impacts, highlighting future research priorities to inform sustainable avitourism management. *Journal of sustainable tourism*: 1-39.

Warakagoda D. and Sirivardana U. (2006). Status of waterfowl in Sri Lanka. In: C. Bambaradeniya, (Ed.). *The Fauna of Sri Lanka*. The World Conservation Union (IUCN), Colombo: 204-215.

Wetlands International (2010). Guidance on Waterbird Monitoring Methodology: Field Protocol for Waterbird Counting. *Wetlands International, Wagenigen, The Netherlands*. 15 pp.

Weerakoon, D, K. and Gunawardena, K. (2012). The Taxonomy and Conservation Status of Birds in Sri Lanka. In: The National Red List 2012 of Sri Lanka; Conservation Status of the Fauna and Flora. Weerakoon, D.K. & S. Wijesundara Eds., *Ministry of Environment, Colombo, Sri Lanka*: 114-133.

Wijesundara, C. S, Chathuranga D, Hettiarachchi T, Perera N, Rajkumar P, Wanniarachchi S, and Weerakoon G. (2015). Avifaunal diversity in Vallai and Thondamanaru areas in

Jaffna: Potential for Ecotourism. Page 302-302 *in* Proceedings of the Peradeniya University International Research Sessions, vol. *19*.

Wijesundara, C. S., D. Warakogoda, U. Sirivardana, D. Chathuranga, T. Hettiarachchi, N. Perera, P. Rajkumar, S. Wanniarachchi, and G. Weerakoon. (2015). Biogeography of water birds in the northern avifaunal region, Sri Lanka. *National Science Foundation, Colombo*: 20.

Wijesundara, C., and P. Rajkumar. (2016). Abundance and status of migratory water birds on Mandaitivu and adjacent areas in Jaffna, Sri Lanka. Pages 324-324 *in* Peradeniya University International Research Sessions. University of Peradeniya, Peradeniya.

Wijesundara, C. S., D. Chathuranga, T. Hettiarachchi, N. Perera, S. Wanniarachchi, and G. Weerakoon. (2016). Population size of the Greater Flamingo in the Jaffna Peninsula, Sri Lanka. Page 80 *in* Postgraduate Institute of Science Research Congress. Postgraduate Institute of Science, University of Peradeniya.

Wijesundara, C, S., Warakagoda, D., Sirivardana, U., Chathuranga, D., Hettiarachchi, T., Perera,N., Rajkumar, P., Wanniarachchi, S., and Weerakoon, G. (2017). Diversity and conservation of waterbirds in the Northern Avifaunal Region of Sri Lanka. *Ceylon Journal of Science 46 (Special Issue):* 143-155.

Wijesundara, C. S., and P. Rajkumar. (2017). Nagar Kovil and Mamunai: Important Refuges for Flamingos and Other Waterbirds in the Jaffna Peninsula. Page 441 *in* Peradeniya University International Research Sessions. University of Peradeniya, Sri Lanka.

Wijesundara, C. S., S. Wanniarachchi, T. Hettiarachchi, S. Galappaththi, A. Weerawardhana, and P. Rajkumar. (2018). Population size and movements of the greater flamingo (*Phoenicopterus roseus*) in the Jaffna peninsula, Sri Lanka: Results from a long-term study. *Ceylon Journal of Science* **47**:373-378.

Antioxidant activity in selected fresh vegetables in Jaffna

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Abstract— Generally, fruits and vegetables are very good source of natural antioxidants which consists of many different antioxidant components. Though there are many vegetables available in the markets with high antioxidant values and high mineral composition, most of them are very expensive. Hence as an alternative source of minerals and antioxidants the possibility of consuming conventional vegetables was taken into consideration in this study. The procedure was carried out to determine the antioxidant activity based on the inhibition of the free radical 2,2'-diphenyl-1-picrilhydrazil (DPPH) in ethanol extract of the okra (*Abelmoschus esculentus* L.), beetroot *(Beta vulgaris* L.*)*, bitter gourd (*Momordica charantia* L.), brinjal (*Solanum melongena* L.), tomato (*Solanum lycopersicum* L.), banana peppers (*Capsicum annuum*), green chili (*Capsicum annuum* L.), carrot (*Daucus carota*), yard long beans (*Vigna unguiculata subsp. sesquipedalis*) and beans (*Phaseolus vulgaris* L.). According to the results, okra had the highest EC_{50} value (9.255 ± 0.130 mg/mL) while beetroot had the lowest EC_{50} value (0.743 ± 0.032 mg/mL) among the tested vegetables. It showed that Okra had the lowest antioxidant activity while beetroot had the highest antioxidant activity. Beetroot got the lowest EC_{50} value which was higher than the standard L-Ascorbic acid (0.005 ± 0.001 mg/mL). EC_{50} of all the tested vegetable samples are significantly different from each other as well as from L-Ascorbic acid. Considering these results, the local vegetables mentioned above also contain high antioxidant activities and can be included in our diet to lead a healthy life.

Keywords— Antioxidants, DPPH method, EC_{50} value, Radicals, Vegetables

I. INTRODUCTION

Usually all fruits and vegetables have many phytochemicals which possess various bioactivities. It includes antioxidants. Consumers can open themselves to more antioxidants through their diet consisting of fruits and vegetables which is extremely easy and best way (Brookie *et al*., 2018). By consuming fruits and vegetables, we can reduce the risk of oxidative damages to cells (Sun *et al*., 2002). Fruits and vegetables are very good sources of natural antioxidants (Justina *et al*., 2013). Hence those are alluded to as "super foods" or "functional foods" (Megan, 2015). These antioxidants are carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione and endogenous metabolites (Justina *et al*., 2013). Phenolics found in fruits and vegetables possess a broad spectrum of biochemical activities such as antioxidant, anticarcinogenic and antimutagenic properties (Nakamura *et al*., 2003 and Tapiero *et al*., 2002). Thus according to the previous researches it has been highly recommended to include proper combination of fruits and vegetables in daily diet, whose phytochemicals synergistically act to reduce the risk of degenerative diseases like cardiovascular disease and cancer (Deepa *et al*., 2015).

Previous studies have shown that the importance of vegetables in a healthy diet and to prevent degenerative diseases that is caused by oxidative stress (Sreeramulu *et al*., 2010). The antioxidant compounds like vitamins and phytochemicals, such as ascorbic acid, carotenoids, polyphenols and fibre have been regarded as the bioactive substances responsible to fight against these effects (Szeto *et al*., 2004). Based on various studies on the antioxidant compounds in several vegetables the aim of current research was focused on determination of antioxidant activity of local vegetables which are less expensive.

II. MATERIALS AND METHODS

A. Vegetables used in this study

Tomato (*Solanum lycopersicum* L. [Solanaceae]), Beetroot (*Beta vulgaris* L. [Amaranthaceae]), Carrot (*Daucus carota* L. [Apiaceae]), Bitter gourd (*Momordica charantia* L. [Cucurbitaceae]), Brinjal (*Solanum melongena* L. [Solanaceae]), Bean (*Phaseolus vulgaris* L. [Fabaceae]), Banana pepper (*Capsicum annuum* [Solanaceae]), Yard long bean (*Vigna unguiculata subsp. Sesquipedalis* L. [Fabaceae]), Okra (*Abelmoschus esculentus* L. [Malvaceae]) and green chilli (*Capsicum annuum* L. [Solanaceae]) were purchased from local farmers in Jaffna. The identification was done by a taxonomist in the University of Jaffna. Fruits of tomato, bitter gourd, brinjal and chili were used in this study. Roots of beet and carrot were used. Seeds were used from banana pepper and pods were used from yard long bean and Okra.

B. Determination of antioxidant activity and EC_{50} values

The procedure was carried out to determine the antioxidant activity (AC) in the formulation which was proposed by Williams *et al*. (1995). It is based on the inhibition of the free radical 2, 2'-diphenyl-1-picrilhydrazil (DPPH) in ethanol extract of the samples. Here a modified version was applied following recommendations by Molyneux (2004).

To evaluate the antioxidant activity in fresh vegetable samples, each sample was taken directly after washing. Each sample (2 g) was taken and ground by using motor and pestle. Then after adding 10 mL of ethanol (96%) it was allowed to stir for 40 minutes at room temperature and then centrifuged for 10 minutes at 10,000 g to retain the supernatant.

The volume of the extract ranged from 0 to 100 µl and mixed in test tubes, with 2 mL of an ethanol solution of DPPH 40 ppm, prepared on the same day under dark conditions. Then

ethanol was added to that until the final volume became 3 mL. After 30 minute incubation in dark at room temperature the absorbance was taken at 517 nm by using a spectrophotometer. The experiment was carried out in three replicates.

The percentage of inhibition is calculated by using the following equation for each extract.

$$\text{Percentage scavenging activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

After plotting the graph (concentration of DPPH solution vs percentage scavenging activity), the EC_{50} values of each sample were determined and antioxidant activity is expressed as mg/mL. Ascorbic acid was taken to draw a standard curve to compare the results instead of vegetable samples.

III. DATA ANALYSIS

Values were expressed as mean ± SD of three replicates. Means were analyzed by Duncan's test using SPSS software (IBM SPSS statistical software).

IV. RESULTS AND DISCUSSION

According to the results okra got the highest EC_{50} value (9.26 ± 0.13 mg/mL) while beetroot got the lowest EC_{50} value (0.74 ± 0.03 mg/mL) among these vegetables. It showed that okra had the lowest antioxidant activity while beetroot had the highest antioxidant activity among these selected vegetables. Okra had required high concentration of the extract to require 50% scavenging of radicals under experimental conditions that had been used while beetroot required low concentration of the extract to 50% scavenging of radicals under experimental conditions that had been used.

Though beetroot got lowest EC_{50} value among these selected vegetables, that EC_{50} value is lower than the standard sample that was L-Ascorbic acid (0.005 ± 0.001 mg/mL). None of the samples showed significantly more or less similar EC_{50} for standard L-Ascorbic acid. All the selected vegetable samples are significantly different from each other as well as from L-Ascorbic acid.

The EC_{50} values reduced significantly ($p < 0.05$) in the following order; *A. esculentus* L. (9.255 ± 0.130 mg/mL) > *M. charantia* L. (6.532 ± 0.83 mg/mL) > *S. melongena* L. (5.785 ± 0.022 mg/mL) > *L. lycopersicum* L. (3.243 ± 0.137 mg/mL) > *C. annuum* (banana pepper) (3.151 ± 0.079 mg/mL) > *C. annuum* L. (chilli) (2.702 ± 0.074 mg/mL) > *D. carota* (1.893 ± 0.018 mg/mL) > *V. unguiculata* L. (1.476 ± 0.095 mg/mL) > *P. vulgaris* L. (1.066 ± 0.074 mg/mL) > *B. vulgaris* L. (0.743 ± 0.032 mg/mL). The results are presented in Table 1 and Figure 1 given below. Conversely DPPH antioxidant activity increased significantly among the vegetables.

The results obtained showed that local vegetable have high antioxidant activities and there is variation in the content. The results regarding antioxidant activities are in par with the previous studies done by Scarano *et al*. (2018) in carrot and Karagyozov *et al*. (2013) and Guldiken *et al*. (2016) in beetroot. Also the antioxidant activity in Okra found in the present research is supported by the previous studies done by Lianmei *et al*. (2014). In this study, a modified DPPH method (Molyneux, 2004) is used to determine the antioxidant activity which is a very common method. Various other methods can also be tried in future studies including processed vegetables as well. The present study reveals that fresh vegetables provide sufficient amounts of antioxidants if they are included in the regular diet.

Table 1: Antioxidant activity of fresh vegetables

Sample	EC_{50} value (mg/mL)
Banana pepper	3.15 ± 0.08 [a]
Beans	1.07 ± 0.07 [b]
Beet	0.74 ± 0.03 [c]
Bitter gourd	6.53 ± 0.08 [d]
Brinjal	5.78 ± 0.02 [e]
Carrot	1.89 ± 0.02 [f]
Chili	2.70 ± 0.07 [g]
Ladies fingers	9.26 ± 0.13 [h]
Yard Long beans	1.48 ± 0.10 [i]
Tomato	3.24 ± 0.14 [a]
L-Ascorbic acid	0.005 ± 0.00026 [j]

Values with different alphabets are significantly different ($p < 0.05$)

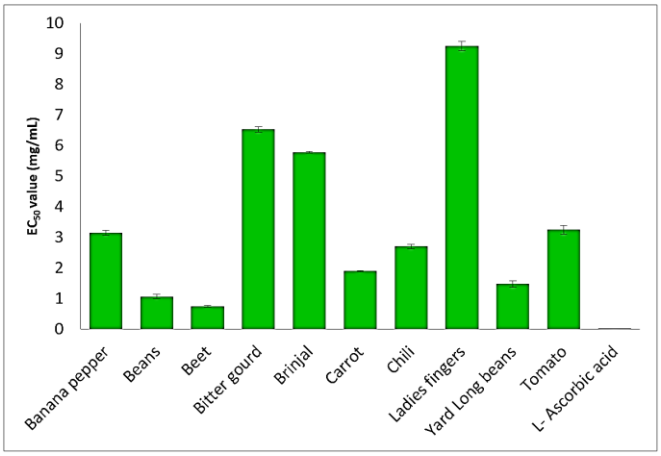

Figure 1. EC_{50} values of fresh vegetables.

V. CONCLUSION

The local vegetables also contain high antioxidant activities. Considering these results, nutritional levels in our diet can be increased by the vegetables that could be grown in our home gardens. Due to the growing demand for organic vegetables at present the current research in developed countries are mainly concerned about increasing the antioxidant content in vegetables by using various combinations of natural fertilizers.

In this research further studies should be done to find the changes in the antioxidant activities in vegetables after processing and consumers should be advised to follow proper cooking methods to avoid the loss of antioxidants during cooking.

VI. REFERENCES

Brand-Williams, W., Cuvelier, M. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm Wissenschaft Technol* 28, 25-30.

Brookie, K. L., Best, G. I and Conner, T. S. (2018). Intake of raw fruits and vegetables is associated with better mental health than intake of processed fruits and vegetables. *Frontiers in Psychology* 9, 487-487.

Deepa, P., Lavanya, B., Sasirekha, G. V. and Santhi, M. (2015). Phytochemical Screening, Antioxidant and Antidiabetic Activity of *Musa acuminata, Citrus sinensis* and *Phyllanthus emblica. American Journal of PharmTech Research* 5 (2), 2249-3387.

Guldiken, B., Toydemir, G., Nur Memis, K., Okur, S., Boyacioglu, D. and Capanoglu, E. (2016). Home- processed red beetroot (*Beta vulgaris* L.) Products: Changes in antioxidant properties and bioaccessibility. *International Journal of Molecular Sciences* 17, 858-858.

Zykevičiūtė-Laugks, L., Česonienė, L. and Karklelienė, R. (2013). The influence of the sample preparation of carrots (*Daucus carota* L. Neptun) on the antioxidant activity and phenolic compounds. *Biologija* 59(2), 187-194.

Karagyozov, V., Denev, P., Kratchanova, M. and Kratchanov, C. (2013). Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments. *Czech Journal of Food Sciences* 31(2), 139-147.

Hu, L., Yu, W., Li, Y., Prasad, N. and Tang, Z. (2014). Antioxidant activity of extract and its major constituents from Okra seed on rat hepatocytes injured by carbon tetrachloride. *BioMed Research International*.

Megan, W. (2015). Antioxidants: Health Benefits and Nutritional Information, http://www.medicalnewstoday.com/articles/301506.php

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Journal of Science and Technology* 26(2), 211-219.

Nakamura, Y., Watanabe, S., Miyake, N., Kohno, H. and Osawa, T. (2003). Dihydrochalcones: evaluation as novel radical scavenging antioxidants. Journal of Agricultural and Food Chemistry 51, 3309-3312.

Scarano, A., Gerardi, G., D'Amico, L., Accogli, R. and Santino, A. (2018). Phytochemical analysis and antioxidant properties. *Agriculture* 8,102-102.

Sreeramulu, M. D. and Raghunath, M. (2010). Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. Food Res. Int. 43: 1017–1020.

Sun, J.1., Chu, Y. F., Wu X. and Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. J Agric Food Chem. 50(25): 7449-54.

Szeto, Y. T., Kwok, T.C. and Benzie, I. F. (2004). Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease. Nutrition. 4(20): 863–866.

Tapiero, H., Tew, K. D., Ba, G. N. and Mathe, G. (2002). Polyphenols: do they play a role in the prevention of human pathologies? Biomed. Pharmacother. 56: 200-207.

Impacts of the Thondaimanaru Barrage Construction on Socio – Economic Status of Fishing Communities in Thondaimanaru Lagoon, Jaffna, Sri Lanka

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Abstract— Present study was conducted to determine the impacts of barrage construction on livelihood status of fishers who involve fishing in Thondaimanaru lagoon. The data related to the socio - economic condition of fishers in the particular area were obtained through a pre - tested structured questionnaire and in - depth guided interviews from July 2016 to January 2017. Randomly selected 25 fishermen were interviewed. The collected data were subjected to descriptive statistical analysis. Survey results indicated that 88% of fishermen are living as nuclear families consisting of 4 - 5 members. Majority of fishermen are Hindus and 92% are married. About 64% engaged in fishing are under age group of 36 - 55. Education is still not prominent among the fishers. Only 84% has primary education. The main occupation in this society is fishing and earned highest monthly income LKR (6000 ± 1000). It was found that installation of barrage plays a crucial role in socio - economic status of fishers of Thondaimanaru lagoon. This barrage severely affected the traditional fishing, amount of fish production and its consequences on their income as well. It was apparent from the current study that these fishermen have poor livelihood status and they need support from government or other organizations to enhance their socio – economic status.

Keywords— Barrage, Livelihood, Socio - economics, Thondaimanaru lagoon

I. INTRODUCTION

The fisheries sector in Sri Lanka plays a vital role in economic and social life by providing direct and indirect employment opportunities for about 560,000 people and livelihoods for more than 2.7 million coastal communities (Fisheries industry outlook, 2016). In 2017, the share of fisheries to the Gross Domestic Production (GDP) of the country was 1.3% and the total fish production of the country was 531,310 metric tons (Central Bank of Sri Lanka, 2017). Socio - economic status is a measure of an individual's or family's economic and social position in relation to others, based on various variables responsible for that like income, education, occupation, family effluence, physical assets, social position, social participation, caste, muscle power, political influence, etc. (Reza *et al*., 2015).

In 1953 barrage with sluice gate was erected to prevent free flow of seawater into lagoon and gradually convert it to freshwater lake (Chitravadivelu, 1978). The installation of barrage plays a crucial role in socio - economic status of fisher folk of Thondaimanaru lagoon where it has thrown the traditional employment of fishermen and reduced the production of fish and their income. Prior to 1953 the lagoon provided employment and a livelihood for about 300 fishermen and their families, who netted approximately 150 tons per year (Chitravadivelu, 1978). But the introduction of the barrage with sluice gate has thrown nearly 2/3 of the fishermen out of their traditional employment and bringing down the production of fish about 35 tons per year and disturbed the ecological equilibrium to such an extent that the fish fauna had been reduced from 47 species during 1967 - 1968 to about 15 species in 1978 and inadequate installation, lack of maintenance and poor operation procedures resulted in malfunctioning of the gates and leakage at barrage and changes in lagoon water salinity (Chitravadivelu, 1978). A baseline study carried out in Thondaimanaru lagoon, stated that presence of four fish species such as *Chanos chanos*, *Hemirhamphus* sp., *Nematalosa* sp. and *Mugil cephalus* and after construction of barrage, reduction of biodiversity was observed due to changes in rainfall pattern , salinity and depth (Piratheepa *et al.,* 2016).

Most of the researchers feel that the conversion of fresh water lagoons shall make very big contribution to 70% - 80% of the total requirement but the side effects of this conversion also has to be analyzed environmentally, socially and economically (Sivakumar, 2013). The lagoon converted as lake affected the livelihood of fishing community by lower their harvest and traditional employment. The small scale fisheries sector is mostly the livelihood occupation of the group of population within the extreme poverty that leads to a serious social, economic and political issue. Due to lack of knowledge in the socio - economic conditions of fishers and fishing communities, leads to poor planning and implementation of various fisheries management programs (Devi *et al.*, 2012 & 2016).

The present investigation is conducted to fulfil the objectives that is to identify the present status of socio - economic and livelihood profiles of involved fishermen and to find out some possible suggestions to improve livelihood status of fishermen.

II. MATERIALS AND METHODS

A. Study area

The present study was carried out at Thondaimanaru (Figure 1) which is located in Jaffna district, Northern Province of Sri Lanka. Its geographical coordinates are 80° 08' E - 80° 29' E and 9° 34' N - 9° 49' N **(**Chitravadivelu, 1978) and covered with 75 km^2 of surface area and 287 km^2 of catchments area (Sivakumar, 2013). Majority of the population is involved in agriculture for income generation while fishing occupies the second place in livelihood activities. The study was carried out for seven month period from July 2016 to January 2017.

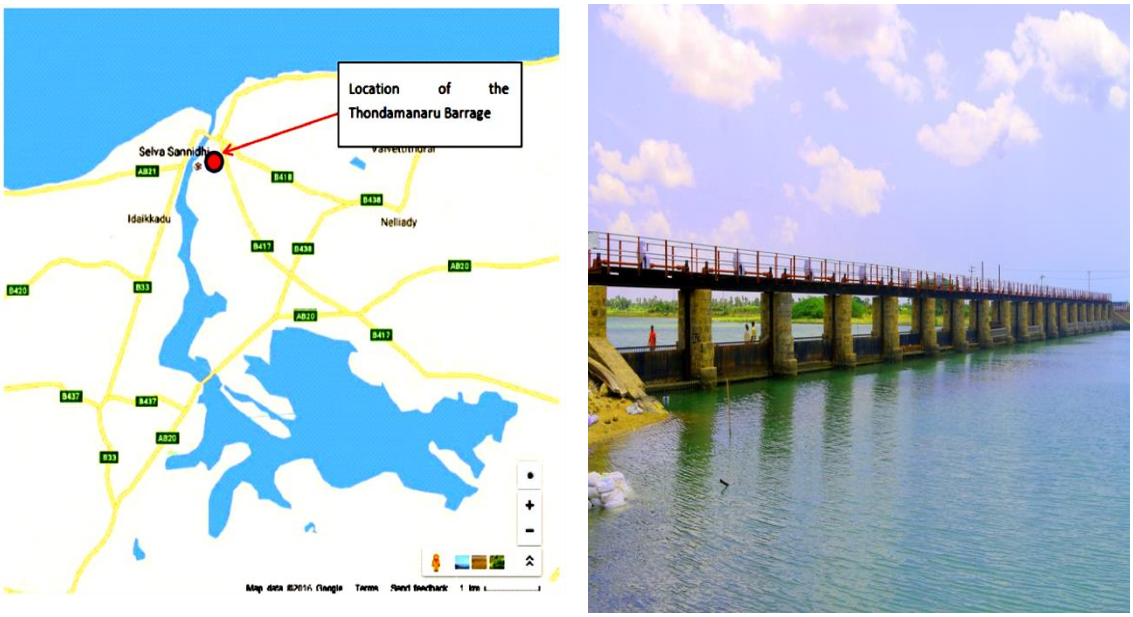

Figure 1. Map Location of barrage (Source: Google Earth) and existing barrage with sluice gate at Thondaimanaru lagoon

B. Data collection

Data were collected in two different methods such as primary and secondary data collection.

1) Primary data collection

The data related to the socio - economic condition of the fisher folk were obtained through structured questionnaire and in - depth guided interviews to understand the fishermen's status earlier and present. Out of 50 active fishermen, a total of 25 were interviewed through field study at Thondaimanaru lagoon and fish market. The socio - economic profiles considered were family type, family size, religion, marital status, gender profile, age, education level, occupation and income. In addition, face to face interviews were carried out. Same questions were repeated for each fisherman. The interview engaged with three sections such as (1) questions related to their production of fishes earlier and after construction of barrage (2) Type of fisheries carried out by them and socio - economic status (3) questions related to loss of fish production and reduction of livelihood. All the participated interviews were informed and the collected data were kept confidential.

2) Secondary data collection

More detailed information related to socio - economic condition of fishers were collected from books, journals, thesis papers, Grama Niladari office and Thondaimanaru fishermen Co - operative society.

C. Data analysis

All the collected data were accumulated and analyzed by using Microsoft Excel analytical tool, version 2013. Results were presented in the form of textual, tabular and graphical forms to know about the present status of livelihood conditions of the fisher folk.

III. RESULTS AND DISCUSSION

The following socio - economic aspects were obtained in detail to show the impact of barrage construction on fisheries.

A. Family Type

Families are classified into two types: (1) Nuclear family: married couples with children and (2) Joint family: group of people related by blood and/or by law. A nuclear family consists of the members of two generations (parents and children) and joint family with members of three or more generations (Hossain *et al*., 2014). Hossain (2014) showed in his studies that 47 families were nuclear whereas only 3 were joint families which suits with the current investigation. In the survey area, it was found that 88% of people belonged to nuclear families and only 12% live as joint family (Figure 2a). In general, changes in lifestyle determine the type of family. But in the study, it was identified that the construction of

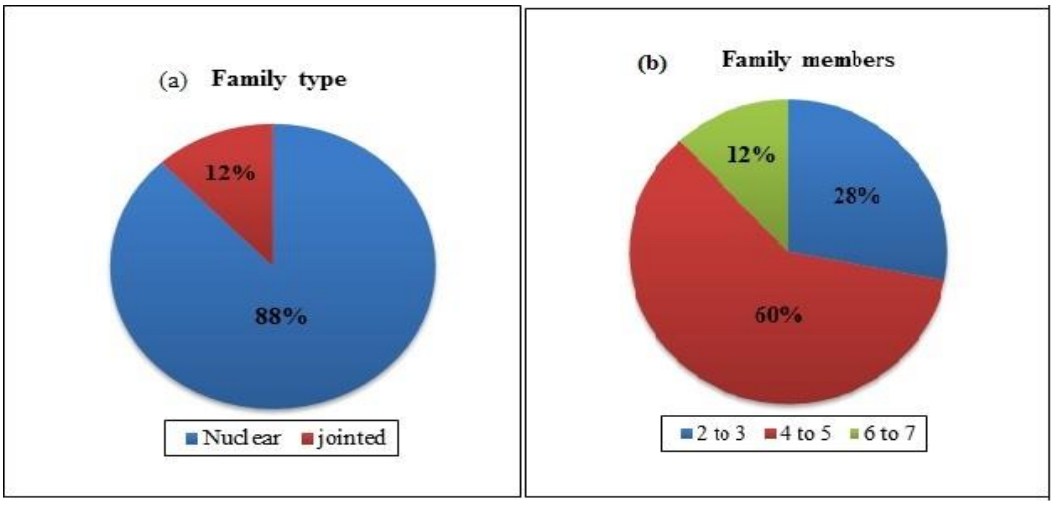

Figure 2. Family type (a) and family size (b) of fishermen of Thondaimanaru lagoon

barrage affected the status of fishing communities by means of their fishing activities and income as well. So they were not willing to have joint family which needs to be more financially staple to run their family.

B. Family size

The family size of fishermen was grouped into 3 categories such as 2 - 3 members (28%), 4 - 5 members (60%) and 6 - 7 members (12%). Most fishermen family belongs to 4 - 5 members (Figure 2b).

C. Religion

In this study it was observed that most of the fishermen are Hindus (72%) and 28% are Christians. Religion is a heritable human capital which plays a very important role in the social and cultural life of people of a particular area and can act as a notable constraint or modifies in social change (Reza *et al*., 2015). The most popular Selva Sannidhi Hindu temple is situated close to the study area and majority of fishermen are Hindus.

D. Marital status

In this survey it was found that, 92% were married and only 8% are unmarried. Majority of past findings highlight that most of active fishermen were married. Married fishermen resulted in a study by Ahamed (1996) which was 94%, match with the present study.

E. Gender profile in Fishing

All the active fishers are male (100%). Fishermen who involved in active fishing were male and female play a passive role in sorting, marketing, look after their household works and support their husband and family.

F. Age structure

The profile age (Figure 3) categorized as 15 - 35 (32%), 36 - 55 (64%) and 56 - 75 (4%). The highest number of fishermen were in between 36 - 55 age groups. Similar study in Bangaladesh, resulted by Ahmed (1996) in Tangail and Ahmed (1999) in coastal region found that 66% and 70% fishermen were less than 40 years age, respectively. There is a negative perception in the society for fishers, which adversely affect the social reputation of the fishermen. In order to get a relief from the depressed mentality, they seek reputed occupations in the society other than fishing. Eventually that leads to low participation of the young in fishing activities (Ragavan *et al*., 2017). Although introduction of barrage severely diminished the amount of fish production and its consequences on their livelihood. Thus that made them less involvement of fishing activities in lagoon.

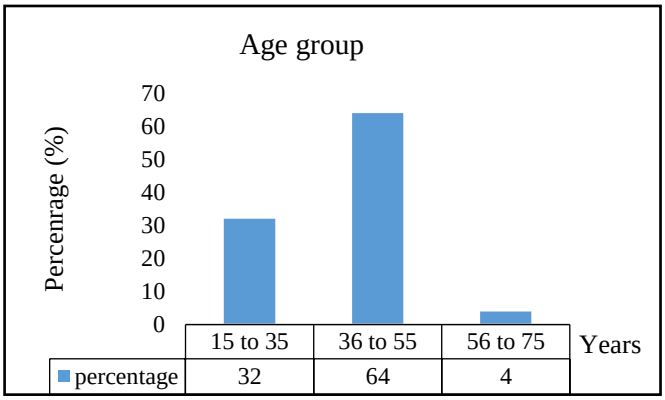

Figure 3: Age structure of Fishermen

G. Literacy rate

There is a strong relationship between social status and education. The higher is the education the better is the livelihood opportunities (Reza *et al*., 2015). Human resource development is largely a function of literacy and educational attainment. Amongst fishermen, literacy and education attainments help to develop conceptual skill and also facilitate the acquisition of technical skill which can have direct bearing on income generation, expenditure and saving activities (Abdullah Al Mamun, 2011). The studies revealed that

majority of fishermen have primary education (84%), 12% of secondary and 4% with tertiary education respectively (Figure 4). Due to low income, lack of awareness and involvement in their traditional occupation may be the reasons for drops in educational status.

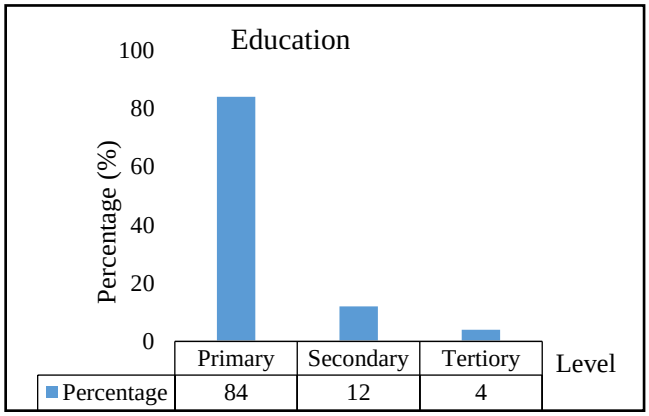

Figure 4: Educational status of Fishermen

H. Occupation

Fishing was the main occupation and 92% of fishermen were involved in fishing and remaining involved in agriculture and wage labour. After erection of northern barrage, more than 250 fisher families sacrificed their fishing opportunities (Chitravadivelu, 1978) and their living subsistence. As a result they have low harvest and income. Even they continue their traditional employment. But some of them shifted to other occupations or migrated to abroad.

I. Income

The fishermen who involved fishing in lagoon adopted traditional fishing methods, such as, cast net, sweep net and diving method. Fishermen use Kaddumaram as their vessel for fishing activity. The monthly Fishing income (Figure 5) in LKR < 3000 (24%), 3000 - 6000 (60%) and > 6000 (16%). The highest fishing income is 3000 - 6000 LKR.

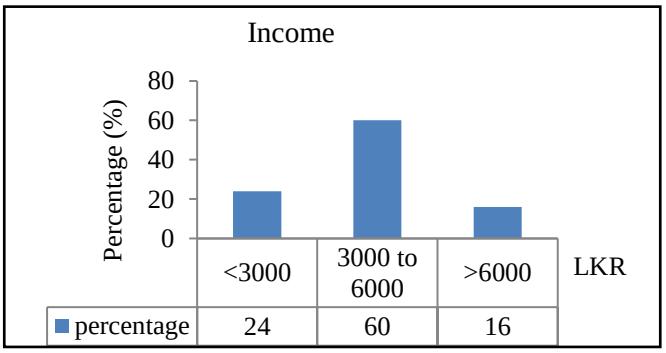

Figure 5: Income of Fishermen

The prime reasons for this condition are loss of production due to reduction of fish and shellfish species, still using the traditional fishing gears and technology, problems in marketing and lack of financial assistance.

J. Reduction in biodiversity

After development of barrage, it is serve as artificial barrier and prevents the recruitment of fish fauna from sea, lessen the yield and changes the water quality parameters of lagoon. Shobiya (2018) in a recent study , stated that the water quality parameters: Water temperature, Electric Conductivity, Total Dissolved Solids, Dissolved Oxygen, salinity and turbidity of Thondaimanaru lagoon significantly ($p < 0.05$) vary with months. Change in lagoon water salinity is an important aspect that affects the availability and composition of aquatic resources and the livelihoods of the people who are depending on these resources (Sugirtharan *et al*., 2014). From the field survey it was found that changes in lagoon water salinity act as a reason for loss of fishery potentialities especially shrimps in Thondaimanaru lagoon where recruitment and development of shrimps get affected and complete depletion of shrimp harvest is observed recent three years, which altered the livelihood of fisher folk.

K. Problems of Fishermen

The following constraints were identified that have been faced by the fishers of Thondaimanaru lagoon:

- Reduction in biodiversity and production of fish and shell fish
- Low income
- Lack of support from government or any organizations
- Lack of awareness
- Following traditional fishing methods
- Lack of marketing facilities and financial assistance

IV. CONCLUSION

The current study revealed that socio - economic status of fishermen of Thondaimanaru lagoon is poor. The socio - economic and livelihood status of these fishermen is not satisfactory because fish production of natural source is declining day by day. Education is still not prominent among the fisher folk. Fishermen get very low income as loss of marine fisheries due to the installation of barrage. Government or other non – governmental organizations should encourage them and step forward to provide hands on training on freshwater fishery and initiate ways to develop freshwater fishery in lagoon by introducing highly valuable fish and shell fishes in the lagoon.

V. ACKNOWLEDGEMENT

We would like to express our heartfelt gratitude to fishers of Thondaimanaru lagoon for their corporation and providing valuable information to successfully complete this research project.

VI. REFERENCES

Abdullah Al Mamun. (2011). Socio-economic condition of fishermen in Kaptai Lake Area. M.Sc. thesis. Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

Ahmed N. U. (1996). Report of the fishermen's socio-economic survey. Fisheries survey and monitoring program. DoF, Tangail, 1 - 4.

Ahmed N. U. (1999). A study on socio-economic aspect of coastal fishermen in Bangladesh. MS Thesis, Dept. Aquacul., BAU, Mymensingh, Bangladesh.

Central Bank of Sri Lanka. (2017). Annual Report.

Chitravadivelu, K. (1978). Fishery Potentialities of the Thondaimanaru Lagoon and a way of developing it, Field Work Center Newsletter 9, 9 - 13.

Devi, N. B. L. Ngangbam, A. K. and Sheela, I. (2012). A study on existing fisheries management system and the problems faced by the fishers in Loktak Lake of Manipur. *Journal of agriculture and veterinary science*, 1(5), 22 - 28.

Devi, N. B. L. Ngangbam and Biswal, N. N., (2016). A review on the current fisheries management system in Manipur with special reference to Loktak Lake. *Journal of agriculture and veterinary science*, 7(4), 63 - 66.

Fisheries Industry Outlook. (2016). Socio – Economic and Marketing Research Division National Aquatic Resources Research and Development Agency.

Hossain, S. A. Z. Z. A. D., Hasan, M. T., Alam, M. T. and Mazumder, S. K., (2014). Socio - economic condition of the fishermen in jelepara under pahartoli of Chittagong district. *J. Sylhet Agril. Univ*, *1*, 65 - 72.

Piratheepa, S., Rajendramani, G. and Eshwaramohan, T., (2016). Changes in fish and shellfishes in Thondamanaru lagoon, Jaffna,Sri Lanka, *Journal of Animal and Vaterinary sciences*, *3*(6), 669 – 673 .

Ragavan, N., Sivashanthini, K. and Sutharshiny, S., (2017). Socio-economics status of fishermen of Allaipiddy village, Jaffna. *Vingnanam Journal of Science*, *12*, 1 - 2.

Reza, S., Hossain, M.S., Hossain, U. and Zafar, M.A. (2015). Socio-economic and livelihood status of fishermen around the Atrai and Kankra Rivers of Chirirbandar Upazila under Dinajpur District. *International Journal of Fisheries Aquatic Studies*, 2, 402 - 408.

Sivakumar, S. S., (2013). Reclamation of Land and Improve Water Productivity of Jaffna Peninsula of Northern Sri Lanka by Improving the Water Quality of the Lagoons. *Research Journal of Science and IT Management 2*, 20 - 27.

Shobiya, P., Sivashanthini, K., Sutharshiny, S., and Gunaalan, K (2018). Variations in important water quality parameters and fish species of Thondaimanaru Lagoon , Jaffna, Sri Lanka, *Vingnanam International Research Conference*, 42.

Sugirtharan, M. Pathmarajah, S. and Mowjood, M. I. M. (2014). Variation of Salinity in Batticaloa Lagoon in Sri Lanka during Wet Season, *Tropical Agricultural Research*, 25(3), 403 – 411